EFFECTS OF SURGERY, ANESTHESIA AND PAIN ON REPRODUCTION AND BEHAVIOUR OF CAPTIVE AND FREE-RANGING DUCKS

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ABSTRACT

Intra-abdominal radio transmitters are used to provide valuable information on reproduction, movement patterns, habitat use, and survival in a variety of wildlife species, including waterfowl. Pain and stress associated during capture, handling, anesthesia, and with instrumentation during surgery may have sublethal consequences, which may interfere with normal behaviour. As pain is produced by any procedure or injury that causes tissue damage, it is likely that waterfowl implanted with radio transmitters would experience pain. Also, placement of transmitters during incubation necessitates an incision into the brood patch which may have significant implications, such as altered incubation patterns resulting in delayed hatch. Therefore this study attempted to quantify the effects of pain and its treatment on captive and free-ranging waterfowl.

Plasma thromboxane B_2 levels indicated that two nonsteroidal anti-inflammatory drugs (flunixin and ketoprofen) may exert pharmacological effects for at least 12 hours in mallard ducks. However, use of flunixin in waterfowl cannot be recommended because of extensive muscular necrosis. Treatment isoflurane-anesthetized mallards with ketoprofen demonstrated a significant analgesic through reduction heart and respiratory rate changes in response to painful stimuli. In free-ranging mallards, longer surgeries were correlated with increased time to first nesting attempt after intra-abdominal transmitter placement. Females that received ketoprofen took 3.5 days less to

the first nesting attempt than females that received saline, indicating that analgesia was beneficial. There was no evidence to suggest that ketoprofen was harmful. Bupivacaine (local anesthetic) may be shorter acting in ducks than in mammals. Sequestration and redistribution of bupivacaine may result in delayed toxicity but mechanisms are unknown. In ruddy ducks, bupivacaine did not appear to achieve long-term analgesia or prevention of post-operative pain-related behaviours. In nesting female mallards, surgery altered incubation patterns in the 24 hour post-operative period, regardless of analgesic (ketoprofen or bupivacaine). Incubation period duration was extended in bupivacaine-treated females compared to ketoprofen-treated females, indicating that analgesia may interfere with brood patch sensation. Increases in corticosterone and progesterone were detected following surgery which may indicate stress and/or pain. The benefits of administering analgesia cannot be overlooked in minimizing effects of placement of radio transmitters on free-ranging waterfowl.

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1. INTRODUCTION

Pain can be difficult to define and can describe a wide variety of sensations that are uncomfortable, unpleasant, irritating, disturbing, severe, intense, distressing, intolerable or disabling which involves more than a single neural mechanism (Woolf 1989). Pain can be classified as two distinct types: physiological pain, which is elicited when an intense noxious stimuli threatens damage to normal tissue (nociceptive pain), and pathological pain, which is the consequence of an "abnormal" state and is associated with tissue damage (Woolf 1987; Woolf 1989). Physiological pain defines a range of transient sensations which serve as protective mechanisms, inducing a "survival" response in the animal (immediate avoidance or learned avoidance responses) minimizing its exposure to potentially harmful stimuli (Clark 1995). Physiological pain can be elicited by mechanical, thermal, or chemical stimuli (Woolf 1989) and can be reduced or removed pharmacologically with the use of analgesics (Danbury 1999). In comparison, pathological pain arises as a consequence of either the inflammatory response that accompanies substantial tissue injury or damage to the nervous system. It may outlast the injury, and can involve spontaneous pain, hyperalgesia (elevated pain response to noxious stimuli), and/or allodynia (pain response to previously innocuous stimuli) (Woolf 1989).

In mammals, the majority of information about pain has been gained from laboratory, companion, and farm animals, and it is recognized that there is a wide range in response to painful stimuli among mammalian species, breeds, and individuals. Information about pain in birds is limited, despite the fact that birds and mammals likely experience pain in a similar manner. Studies indicate that birds possess the neurological components to respond appropriately to painful stimuli (Jones et al. 1985; Willis et al. 1979), have endogenous antinociceptive mechanisms to modulate pain (Bayon et al. 1980; Csillag et al. 1989; Reiner et al. 1984), and treatment with pharmacological agents used in mammals modulates pain pathways and behavioural responses to painful stimuli (e.g. Curro et al. 1994; Danbury et al. 1997; Glatz et al. 1992; Paul-Murphy et al. 1999). However, many animals that may be preyed upon are less likely to display overt pain-associated behaviour because species it may attract attention of predators (Livingston 1994). Changes in avian physiology, such as heart rate, mean arterial pressure, respiratory rate and tidal volume have been used as indications of acute pain (Gentle and Hunter 1990) but these are difficult to measure in conscious animals and can be influenced by stress. In conscious birds, pain can be recognized by changes in posture (guarding of the affected area), temperament, or normal behaviour (i.e. reduced feeding or activity) (Jenkins 1993). Pain may also alter normal responses of a bird to its environment, which may increase susceptibility to predation. Modification of normal behaviour may result in erroneous interpretation of data if unrecognized subtle changes occur in the physiology or behaviour of the bird (Houston and Greenwood 1993). Trauma from surgery could cause pain and alter behaviour, but this has not been investigated in a field setting.

Stress responses may influence the response to noxious stimulation. Separation from social companions can alter pain perception and result in stress–induced analgesia in gregarious species (Frohm and Wallnau 1983; Jones and Harvey 1987). Birds held and tested in familiar pens demonstrate significantly less pain-coping behaviour compared to birds tested in novel pens (Sufka and Hughes 1990; Sufka and Weed 1994). A wide variety of repeated stressful or painful stimuli can induce short-lasting reductions in responsiveness to noxious stimuli (Gentle et al. 1989; Sufka and Hughes 1990). Therefore, stress must be considered a confounding factor in the study of pain, especially in animals not habituated to human intervention.

Surgically induced stress responses are evoked by nociceptive afferent activity induced by tissue damage and manipulation, even in patients that are receiving adequate general anesthesia (Benson et al. 2000). Analgesics decrease the stress response and are most effective when administered pre-emptively (Woolf and Chong 1993). Attenuation of the stress response through adequate pain relief may result in improved healing and patient outcome (Benson et al. 2000). In avian species, corticosterone is the principal stress corticosteroid in birds (Holmes and Phillips 1976) but progesterone has also been identified as an indicator of stress in avian species (Al-Ankari 1998; Gratto-Trevor et al. 1991). Corticosterone, cortisol, catecholamines, and other neuroendocrine assays have been used in an attempt an indication of pain (Cambridge et al. 2000; Smith et al. 1999).

Analgesia can be defined as the relief of pain without loss of consciousness. Analgesics function by decreasing the stimulation of ascending spinal pathways or by activating endogenous descending pain modulation pathways (Clyde 1994). Analgesics,

such as opioids (e.g. morphine) or alpha$_2$-agonists (e.g. xylazine) could be used to provide analgesia to animals receiving intra-abdominal radio transmitters but residual sedation (Plumb 1995) may preclude their use in free-ranging waterfowl because of compromised survival. Local anesthetics and nonsteroidal anti-inflammatory drugs (NSAIDs) do not have sedative effects (Plumb 1995) making NSAIDS potentially more useful in providing operative and post-operative analgesia to free-ranging waterfowl undergoing implantation of radio transmitters.

In avian species, NSAIDs and local anesthetics have been recommended for treating and preventing pain (Ritchie and Harrison 1994) but the pharmacodynamics and pharmacokinetics of these agents in birds are unknown. Nonsteroidal anti-inflammatory drugs produce anti-inflammatory, analgesic, antipyretic, and antithrombotic actions by inhibition of prostaglandin production. More specifically, NSAIDs block access of arachidonic acid to its binding site on the cyclo-oxygenase enzyme thus preventing conversion to thromboxane B_2 (TBX) (Vane 1971). Plasma levels of NSAIDs do not reflect physiological or pharmacological activity (Owens et al. 1995) as NSAIDs are weak acids (Landoni et al. 1995), are highly protein bound, and tend to accumulate in areas of inflammation (Lees et al. 1987). Studies have shown a strong correlation between the actions of NSAIDS on thromboxane B_2 in the plasma and prostaglandin E_2 in the exudate for both ketoprofen and flunixin (Landoni et al. 1995; Toutain et al. 1994). Since the inhibition of PGE_2 in inflammatory exudate is a significant component of the clinical effect of NSAIDS, it is not unreasonable to attempt to equate TBX concentration with clinical response. However, not all clinical effects correlate well with

eicosinoid suppression, and central effects on analgesia may also be significant (Dirig and Yaksh 1997).

Local anesthesia before tissue trauma can significantly reduce postoperative pain as it prevents nociceptor sensitization and therefore avoids central changes that are secondary to activation of pain pathways (Coderre and Melzack 1987). In avian species, local anesthetics are often used without sedation or general anesthesia for minor surgical procedures, and for operative and/or post-operative pain relief in conjunction with general anesthesia for more involved procedures (Paul-Murphy and Ludders 2001). In domestic chickens, bupivacaine has produced effective analgesia in two pain models (Glatz et al. 1992; Hocking et al. 1997). Birds may be more sensitive to toxic effects of local anesthetics, as lower doses (2.7 - 3.3 mg/kg) (Hocking et al. 1997) are required to produce toxic effects compared with dogs (3.5 -4.5 mg/kg) (Skarda 1996). However, pharmacokinetics of local anesthetics have not been determined in avian species.

This study was undertaken to gain more insight into the mechanisms and sublethal effects of pain and stress in an avian model, the duck. Wildlife managers often rely on information obtained from radio telemetry studies for their management decisions. Transmitters are used to provide valuable information on reproduction, movement patterns, habitat use, and survival in a variety of wildlife species, including waterfowl. Intra-abdominal transmitters are often used in waterfowl preferentially over externally-mounted transmitters (Korschgen et al. 1984) as they appear to have less effect on normal behaviour (Greenwood and Sargeant 1973; Pietz et al. 1993) and reproductive effort (Paquette et al. 1997; Pietz et al. 1993; Rotella et al. 1993). Pain and

stress associated during capture, handling, anesthesia, and with the instrumentation during surgery may have sublethal consequences, which can interfere with normal behaviour. Disruption of normal behaviour may lead to lowered productivity and survival of ducks, decreased sample size and loss of transmitters (Heusmann et al. 1978; Mickelson 1975; Moseley and Mueller 1975; Rotella and Ratti 1990).

Placement of an intra-abdominal transmitter necessitates general anesthesia and surgery. Isoflurane is usually used for anesthesia during transmitter placement but traditionally supplemental analgesia has not been provided (Korschgen et al. 1984; Olsen et al. 1992) even though isoflurane does not provide operative or post-operative analgesia (Dohoo 1990). As pain is produced by any procedure or injury that causes tissue damage (Kanjhan 1995), it is likely that waterfowl implanted with radio transmitters would experience pain. Also, placement of the transmitter during incubation necessitates an incision in the brood patch which may have significant implications, such as altered incubation patterns resulting in delayed hatch..

The thesis begins with a literature review in chapter 2 which outlines pain physiology, alteration of pain perception in response to stress, and control of pain in avian species. Where applicable, comparisons are made to mammals. The six main parts of this study are outlined and examined in chapters 3 to 8. The final chapter consists of a general discussion of the effects of pain, stress and analgesia on ducks. In addition, direction of future research is addressed. Chapters 3 and 4 describe the pharmacodynamics of two NSAIDS (flunixin and ketoprofen) and pharmacokinetics of bupivacaine in mallard ducks (*Anas platyrhynchos*), respectively. The efficacy of these

agents were examined in chapters 5 through 8 with an investigation of physiological responses, reproductive indices and behaviour. In chapter 5, ketoprofen analgesia was assessed in isoflurane-anesthetized mallards compared to saline injected controls through non-invasive methods by measuring heart rate, respiratory rate, duration of stimulus required to induce gross purposeful movements. In this study, the potential confounding influence of stress on these parameters was removed by anesthetizing the ducks.

During capture, handling and placement of intra-abdominal transmitters, a bird likely experiences stress and pain. To examine this more closely, physiological parameters, subsequent nesting success and short-term survival were determined in free-ranging ruddy ducks (*Oxyura jamaicensis*) after isoflurane or propofol anesthesia with bupivacaine local anesthetic to implant a radio transmitter (chapter 6). The effectiveness of bupivacaine analgesia was tested by comparing post-operative behaviour of ruddy ducks implanted with radio-transmitters with those that did not have surgery. In another study (chapter 7), long-term effects and safety of pre-emptive ketoprofen was examined by comparing its effects on post-operative nesting success and survival of female mallard ducks implanted with radio-transmitters with those given saline. Finally, the impacts of surgery and stress on incubation was examined in nesting female mallards where bupivacaine and ketoprofen analgesia were compared.

Estimates of duckling survival rates are critical for effective waterfowl population management. Waterfowl broods are difficult to monitor because of high mobility (Dzus and Clark 1997; Rotella and Ratti 1992) and low visibility (Ringelman and Flake 1980). Females often are radio-marked and followed to give an estimate of

duckling survival (Rotella and Ratti 1992). More accurate measurements of timing and causes of duckling mortality may be achieved by radio marking individual ducklings within the brood (Korschgen et al. 1996; Mawhinney and Diamond 1999) However, transmitters may have deleterious effects, making wild ducklings more susceptible to mortality from chilling (Bakken et al. 1996; Bakken et al. 1999), exhaustion (Talent et al. 1983), predation (Mawhinney and Diamond 1999; Talent et al. 1983) and disease (Mendenhall and Milne 1985). Captive studies report nil to little effect of transmitters but ducklings in these studies were maintained in ideal conditions (Davis et al. 1999; Mauser and Jarvis 1991; Zenitsky 1993). To obtain a better insight into this problem, the lethal and sublethal effects of radio transmitters on mallard ducklings exposed to natural weather conditions are investigated (Appendix).

2. LITERATURE REVIEW

2.1 Avian Pain

2.1.1 Physiology

Birds represent the most abundant and diverse class of air-breathing vertebrates consisting of approximately 9700 species. However, practitioners encounter only a few species from selected orders which include: anseriformes (i.e. ducks, geese, swans), columbiformes (pigeons and doves), galliformes (i.e. chickens, turkeys, pheasants), ratites (i.e. ostriches, emus), falconiformes (diurnal birds of prey, i.e. eagle, hawks falcons), strigiformes (i.e. owls), psittaciformes (i.e. parrots), piciformes (i.e. toucans), and passeriformes (i.e. perching birds, finches, canaries) (Lancaster 1997). In mammals, the majority of information about pain has been gained from laboratory, companion, and farm animals, and it is recognized that there is a wide range in response to painful stimuli among mammalian species, breeds, and individuals. Information about pain in birds is limited and it has been derived from only a handful of species from the anseriformes (Gottschaldt et al. 1982; Leitner and Roumy 1974), columbiformes (Bayon et al. 1980; Necker and Reiner 1980), galliformes (Gentle 1997; Gentle and Hunter 1990; Hocking et al. 1997; Hughes et al. 1992), and psittaciformes (Curro 1994; Paul-Murphy et al. 1999).

It is generally accepted that birds perceive pain in a similar manner to mammals. Birds possess the neurological components to respond appropriately to a painful stimulus (Jones et al. 1985; Willis et al. 1979), have endogenous antinociceptive mechanisms to modulate pain (Bayon et al. 1980; Csillag et al. 1989; Reiner et al. 1984), and treatment with pharmacological agents used in mammals modulates pain pathways and behavioural responses to painful stimuli e.g. (Curro et al. 1994; Danbury et al. 1997; Glatz et al. 1992; Paul-Murphy et al. 1999). Pain perception allows an animal to minimize its exposure to potentially harmful stimuli (Clark 1995). However, many animals that may be preyed upon are less likely to display overt pain-associated behaviour because species it may attract attention of predators (Livingston 1994). Considerable variation in behavioural responses to pain may occur among avian species, breeds, strains, or individuals and there is no reliable or universal indicator of pain (Gentle 1992). However, most practitioners are able to recognize acute, severe pain but chronic pain may go undetected, especially if the practitioner is unfamiliar with the normal behaviour of the species. It is therefore advisable to treat for pain when dealing with conditions known to be painful in humans (Flecknell 1988).

The purpose of this review is to provide information on pain physiology and related behaviour that may lead to better understanding of pain mechanisms, and therefore, insight into appropriate analgesic therapies. However, interpretation of experimentally produced measures of pain reflexes can be problematic because they may not represent a measure of true pain behaviour (Chapman et al. 1985).

2.1.1.1 Pain Pathways

A noxious stimulus is defined as a potentially tissue damaging stimulus and a receptor sensitive to a noxious or potentially noxious stimulus is termed a nociceptor. A polymodal nociceptor is a pain receptor that has more than one function (Gentle 1992). The physiology of pain involves two processes: (1) a peripheral process involved with detection and transmission of information concerning potential tissue damage and (2) a central process governing the cerebral response to this information (Kanjhan 1995).

2.1.1.1.1 Peripheral Nervous System

Three types of nociceptors have been identified in birds: high threshold mechano-thermal, mechanical, and thermal nociceptors. The mechano-thermal (polymodal) nociceptors that respond to thermal and mechanical stimulation have been identified in pigeons (Necker and Reiner 1980), ducks (Leitner and Roumy 1974), and chickens (Gentle 1989). Receptors respond to both mechanical stimulation and temperatures above 40 °C (Gentle 1989; Necker and Reiner 1980). Conduction is very slow and is likely comparable to mammalian unmyelinated C-fibers, and probably arise from cutaneous free nerve endings. Increasing the stimulus magnitude results in an increase in the number of responses. Some fibres show continuous response up to the highest temperature tested (56 °C), whereas, other fibers show a clear peak in response at a lower temperature and increasing stimulus intensity beyond this temperature results in a reduced response (Gentle 1991; Gentle 1989).

High threshold mechanical nociceptors are likely equivalent to myelinated Aδ- and unmyelinated C-fibers originating from cutaneous free nerve endings, and have been reported in chickens (Gentle 1989; Holloway et al. 1980) and waterfowl (Gottschaldt et al. 1982; Gregory 1973). Receptive fields are similar to polymodal nociceptors but some have larger receptive fields. Increasing the stimulus strength produces an increase in response which may be either linear or exponential. In response to a maintained mechanical stimulus, receptors adapt rapidly at low stimulus intensities but the length of response increases with increasing stimulus magnitude (Gentle 1991).

Thermal nociceptors, without mechanical sensitivity, respond in a similar manner as the polymodal nociceptors (Breward and Gentle 1985). They are either Aδ- or C-fibers (Gentle 1989) and have been reported in pigeons (Necker and Reiner 1980) and chickens (Gentle 1992). These receptors appear to be less sensitive to cold than the corresponding receptors in mammals (Leitner and Roumy 1974). While threshold of heat nociceptors tend to be higher in avian species compared to mammals, it is not surprising as body and skin temperature are higher (41 to 42 °C) in birds (Necker and Reiner 1980). However, when comparing the physiological responses of nociceptors found in the chicken with those found in mammals, discharge patterns, and receptive field size are very similar (Beitel and Dubner 1976; Bessou and Perl 1969; Holloway et al. 1980; Torebjork et al. 1984).

Tissue injury and acute pain can effect both peripheral and central nervous systems in mammals, and alter sensitivity to subsequent stimuli. This sensitization may be characterized by: 1) a lower threshold of activation, 2) an increased response to

noxious stimuli, 3) a shorter response latency, 4) a longer duration of response to stimulation (persistent pain), 5) an increased response to a given stimulus intensity or spontaneous activity, 6) and/or spread of pain and hyperalgesia to uninjured tissue (Campbell and Meyer 1983). Central sensitization occurs when threshold required to activate dorsal horn neurons decreases following activity of nociceptors. Changes in receptive field properties are caused by the recruitment of previously subthreshold components as a result of increased synaptic output or increased excitability of the postsynaptic cell (Woolf and Thompson 1991). Phosphorylation of receptors may be a key feature of central sensitization induction (Chen and Huang 1992).

Peripheral sensitization occurs when nociceptors become responsive to a wider range of stimuli (Sosnowski et al. 1992) or when other receptors respond abnormally to a sustained stimuli of another type (Neugebauer and Schaible 1988). Prostaglandins (PGs) play an important role in pain modulation in both mammal (Pateromichelakis and Rood 1982) and avian species (Clark 1995; Nicol et al. 1992) by lowering activation threshold during tissue injury and inflammation (Nicol et al. 1992). Direct action of eicosinoids, PGE_1 and PGE_2, sensitize small-diameter sensory nerve fibers to thermal and mechanical stimulation (Pateromichelakis and Rood 1982). In avian sensory neurons, exposure to PGE_2 produces a dose-dependent increase in the release of substance P (SP) through inward flux of calcium (Nicol et al. 1992). Substance P appears to be an important mediator of components of the inflammatory response, especially neurogenic inflammation (Payan 1989).

2.1.1.1.2 Central Nervous System

As in mammals, pain signals in birds are transmitted from peripheral receptors to several areas of the midbrain and forebrain by multiple ascending spinal pathways (Pateromichelakis and Rood 1982). The spinal cord dorsal horn of chickens is arranged into six laminae which can be distinguished on the basis of cell size and distribution (Holloway et al. 1980). Nociceptive information is transmitted to the lamina I and outer lamina II of the dorsal horn via Aδ and C primary afferent fibers (Willis 1988). Substance P is an important neurotransmitter of the nociceptive inputs to the spinal cord (Otsuka and Yanagisawa 1990). In birds, the distribution of the neurons is similar to that of the nociceptive spinothalamic tract cells in the monkey (Willis et al. 1979) and cat (Jones et al. 1985). The neurons receive inputs from substance P-containing axon terminals which also appear to play an important role in neurotransmission of pain in birds (Zhai and Atsumi 1997). One study examining the 2-[^{125}I]iodomelatonin binding sites in chicken spinal cord, suggests that melatonin may also be important in transmission of sensory information such as pain signals. The distribution of [^{125}I]iodomelatonin within the dorsal gray horns was similar to those of substance P and opiate receptors (Wan and Pang 1994). In chickens, α-2 receptors have been identified in the brain. Distribution of these receptors have been mapped and closely resemble patterns seen in mammals (Danbury 1999; Danbury et al. 1998; Danbury et al. 1998).

Little is known about pain inhibiting systems of the bird but opioids have been identified in the central nervous system (Bayon et al. 1980; Csillag et al. 1989; Reiner et

al. 1984). Birds possess μ-, κ- and δ-opioid receptors in similar proportions as seen in man (Danbury et al. 1998). As with mammals, endogenous opioid systems appear to modulate central processing of noxious information in birds. Opioid receptors are detectable in chick embryos from the age of 10 days (Hendrickson and Lin 1980) and are concentrated in areas that are thought to play key roles in sensory input processing and memory (Csillag et al. 1989). Within the avian telencephalon, the distribution of β-endorphin and enkephalin-like immunoreactivity, is similar to the mammalian telencephalon (Bayon et al. 1980; Reiner et al. 1984). Broiler breeding male chickens with degenerative joint disease affecting the hip, walked more slowly when they were given an injection of naloxone (an opioid antagonist). As in mammals, this evidence demonstrates that the endogenous opioid system plays and important role in pain modulation and perception (Hocking et al. 1999).

2.1.1.2 Evaluation of Pain and Pain Associated Behaviour

Recognition of pain and anxiety in animals is critical for appropriate analgesic selection and pain relief. Experimental evidence shows that there is a relationship between activation of nociceptors and display of behavioural evidence of pain (Gentle 1992; Gentle 1997; Gentle and Thorp 1994; Gentle and Tilston 1999). Nociceptive stimuli that have been used to investigate pain in birds include electric shock (Bardo and Hughes 1978; Paul-Murphy et al. 1999), comb pinch (Woolley and Gentle 1987), feather removal (Gentle and Hunter 1990), cutaneous thermal stimulation (Hughes 1990;

Hughes et al. 1992; Paul-Murphy et al. 1999; Woolley and Gentle 1987), injection of intra-articular sodium urate (Gentle 1997) or formalin (Hughes and Sufka 1991), and oral presentation of algogenic (pain producing) substances (Gentle and Hill 1987; Szolcsanyi et al. 1986). Although there are no reliable or universal indicators of pain, birds tend to respond to noxious stimuli with a flight-fight response (escape reactions, vocalizations, and excessive movement) (Stoskopf 1994) and/or conservation-withdrawal responses (Woolley and Gentle 1987). The conservation-withdrawal system is typified in chickens by crouching immobility after noxious cutaneous thermal stimulation. Noxious thermal stimulation produces activity primarily in the unmyelinated C-fibre polymodal nociceptors and this receptor activation is most likely to initiate this type of behaviour in domestic fowl (Gentle et al. 1989).

In chickens, acute pain (such as feather removal) is usually characterized by decreased head movements, increased heart and respiratory rates, and increased blood pressure. The magnitude and duration of the response depended on the strength of the stimulus (Gentle and Hunter 1990). Electric shock or comb pinch producing acute pain results in active avoidance behaviour involving vigorous escape attempts (jumping and wing flapping) with some vocalization (Paul-Murphy et al. 1999). Feather removal can produce either active or passive behavioural changes. After removal of a single feather, birds become agitated with wing flapping and/or vocalization. In comparison, inappetence, inactivity and "puffed-up" appearance are often demonstrated when birds are exposed to prolonged pain (Gentle and Hunter 1990). Continual feather removal tends not to produce an exaggerated escape response; instead birds crouch in an

immobile state and electroencephalogram (EEG) shows a characteristic high amplitude low frequency activity similar to that seen during sleep (Tobler and Borbely 1988; Van Luitelaar et al. 1987) or catatonic states (Gentle et al. 1989; Ookawa 1972).

Immobility is a complex behavioural reaction to a painful (feather removal) (Gentle and Hill 1987; Gentle et al. 1989) and/or fear-inducing (restraint) (Gentle et al. 1989; Hughes and Sufka 1990) stimulus. Procedures designed to increase fear, prolong the immobility reaction in chickens, while procedures that reduce fear, attenuate the response (Gentle et al. 1989; Jones 1989). Immobility may be an evolutionary anti-predator strategy to prevent further damage produced by struggling and allow escape should the occasion arise. During continual feather removal, birds respond by displaying crouching immobility. It is unknown if the animal feels pain but changes in blood-pressure and EEG arousal immediately after feather removal suggest pain sensation is present (Gentle et al. 1989). The functional significance of this change in behaviour from active escape to crouching immobility may also be related to learned helplessness. This behavioural pattern develops when an animal experiences traumatic events which are aversive and continue to occur despite attempts by the animal to reduce or eliminate them (Gentle and Hunter 1990). Studies in mammals suggests that learned helplessness may give rise to analgesia (Hutson et al. 1984).

Other nociceptive tests produce immobility. Chemicals causing pain in human dermal lesions, affect birds with oral lesions (Gentle and Hill 1987). In the human blister-base test, acetylcholine (ACh) causes pain that is reported to have immediate onset and brief duration (15 to 65 seconds) (Armstrong et al. 1953.). Chickens show

passive immobility without escape attempts in response to ACh. The response was also immediate and lasted for 1 minute or less (Gentle and Hill 1987).

Thermal trauma such as partial beak amputation in the chicken, involving cutting and cautery, results in full thickness burns. The pain experienced at amputation results from massive injury discharge in the nerve fibres lasting approximately 15 seconds. However, after the initial discharge, no abnormal pattern of response to cutaneous stimulation was detected in any of the sensory receptors in the beak stump for 4.5 hours after amputation. The absence of change in peripheral neural input following beak amputation may suggest an absence of pain during this period (Gentle 1991). A similar pain-free period has also been observed in human patients following full-thickness burns (Robertson et al. 1985). Normal beak usage after amputation is present for approximately 6 hours after amputation, but by 24 hours after amputation chickens were less mobile, unwilling to peck at the environment, and had decreased food and water intake (Duncan et al. 1989). More detailed studies of beak usage after amputation demonstrate guarding behaviour and hyperalgesia, where birds had significant reductions in environmental pecking, preening, beak wiping and head shaking which persisted for 6 weeks after surgery (Gentle 1991; Gentle et al. 1990). In one study, inactivity was observed as long as 56 weeks after surgery (Eskeland 1981). These responses, as well as, altered food intake, reduced weight gain and egg production (Duncan et al. 1989; Gentle et al. 1982; Glatz 1987; Hargreaves and Champion 1965) provide evidence for chronic pain in birds. Decreased activity is also common in humans suffering from chronically painful conditions (Kauppila 1998).

In addition to behavioural evidence, anatomical and physiological evidence support chronic pain following partial beak amputation. Adjacent to the scar tissue, damaged and regenerating nerve fibers formed extensive neuromas (Breward and Gentle 1985, Gentle, 1986). Electrophysiological recordings from nerve fibres innervating these neuromas are abnormal for trigeminal afferent fibres. The most characteristic abnormality was the presence of abnormal spontaneous neural activity in the trigeminal nerve from the beak stump from 5 to 83 days after initial amputation (Breward and Gentle 1985). Neural activity arising from trigeminal neuromas was similar to that reported in experimental neuromas in the rat (Govrin-Lippmann and Devor 1978; Wall and Gutnick 1974), mouse (Scadding 1981), and cat (Blumberg and Janig 1984). Studies on peripheral nerve injury and subsequent neuroma formation in mammals have suggested that abnormal activity from regenerating axons is implicated in post-amputation stump pain (Seltzer et al. 1991).

Response to noxious stimuli can vary greatly from species to species. Electric shock and thermal tests have been used effectively in gallinaceous birds but one study in conscious parrots found that the large variation among responses prevented meaningful quantitative assessment of temperature threshold (Paul-Murphy et al. 1999). In comparison, it was possible to identify when some birds became aware of the electrical stimulus, because they would look down at their foot or chew on the wire, yet they tolerated higher amounts of electrical stimulus until they would lift their foot. The amount of stimulus that caused the bird to lift its foot could be measured reliably and response was attenuated with opioids (Paul-Murphy et al. 1999).

2.1.1.2.1 Stress-induced Analgesia

Separation from social companions can alter pain perception in species that live normally in groups (Frohm and Wallnau 1983; Jones and Harvey 1987). The two most common indications of social separation in domestic chicks are distress vocalizations and stress-induced analgesia (Sufka and Hughes 1991). Birds may also display ventral recumbency posturing and hyperthermia. When held and tested in familiar large pens, birds showed significantly less pain-coping behaviour compared to birds tested in novel pens (Sufka and Hughes 1990; Sufka and Weed 1994). Under novel conditions, birds behave as normal, alert birds and attentional mechanisms are pre-occupied with exploring a new physical and/ or social environment (Gentle and Corr 1995). A wide variety of repeated stressful or painful stimuli induce short lasting reductions in responsiveness to noxious stimuli termed stress-induced analgesia (Gentle et al. 1989; Sufka and Hughes 1990).

In mammals, there are two theories which describe the role of endogenous opioids in social separation. One theory suggests that opioid system activity is stimulated by stressful experiences such as social separation (social separation-opioid stimulation) (Kehoe and Blass 1986), whereas, another theory suggests the opposite, that social isolation places the animal into a state similar to opioid withdrawal (social separation-opioid withdrawal) (Panksepp et al. 1978). Birds that become separated from conspecifics elicit distress vocalizations in an attempt to reestablish social contact (Gallup and Suarez 1980). This isolation causes a state of endogenous opioid

withdrawal leading to a disinhibition of distress vocalizations while the presence of social companions stimulates the release of endogenous opioids, inhibiting vocalizations (Watson and Sufka 1996). Opioid agonists tend to decrease distress vocalizations whereas opioid antagonists increase separation-induced distress vocalization (Panksepp et al. 1978; Sufka and Weed 1994). However, when morphine was administered to isolated chicks, there was no change in response to thermal nociception or core body temperature (Sufka and Weed 1994). Adrenergic, cholinergic, dopaminergic, GABAergic and serotonergic manipulations produce only modest effects on distress vocalization (Panksepp et al. 1978). These results suggest that some separation-stress behaviours are mediated by opioid systems (i.e., distress vocalization) while others are mediated by nonopioid systems (i.e. stress-induced analgesia and stress-induced hyperthermia) (Sufka and Weed 1994; Watson and Sufka 1996).

Benzodiazepine agonists can modulate stress in many animal models (Muhammad and Kitchen 1994) and thus influence the results of nociceptive tests while having no effect on nociception itself (Rosland et al. 1987). The benzodiazepine agonist, chlordizepoxide (CDP) reverses distress vocalizations in the chick social-separation. As with morphine, CDP reverses distress vocalizations, but unlike morphine, CDP also reverses stress-induced analgesia. It appears that the benzodiazepine is less behaviour specific than morphine in modulating separation-stress behaviours in chicks (Watson and Sufka 1996).

2.1.1.2.2 Analgesia Produced Through Changes in Attention

Pain associated with trauma and disease can be chronic and often involves inflammation. The behavioural responses to tonic pain are complex and pain-coping behaviour can be influenced by changes in the motivational state of the animal. In chickens, changes in attention can produce significant pain suppression and reduction in lameness during experimentally induced tonic pain stimulus (sodium urate) (Gentle and Corr 1995; Wylie and Gentle 1998). Injection of sodium urate produces an acute synovitis that sensitizes joint C-fibre nociceptors and clear clinical measures of inflammation: swelling and an increase in skin temperature over the affected joint (Gentle 1997) Hypoalgesia can be produced by diversion of attention in situations designed to increase feeding motivation or motivation to explore (Gentle and Tilston 1999; Wylie and Gentle 1998). Complete analgesia or marked hypoalgesia was observed in birds deprived of food for 16 hours and then given access to food following sodium urate injection. This analgesia could be completely reversed by naloxone which suggests that this analgesia may be opioid mediated (Wylie and Gentle 1998). Distraction and attention focussing strategies have been used to help human patients cope with chronic low-level pain. Coping is based on the cognitive action of switching attention, thus when patients were fully engaged in a task, they were not processing pain at the same time (Eccleston 1995). The absence of pain-related behaviour does not necessarily indicate an absence of pain. Similar to humans, expression of pain can be altered by the motivational state of the individual (Gentle and Corr 1995) but the analgesia experienced during this is likely only temporary.

Although the precise mechanism is unknown, there is evidence that the peripheral nervous system plays a significant role in inflammation (Gentle and Hunter 1993). Some evidence suggests that changes in attention, resulting in a reduction of pain, may also have a direct influence on inflammation, leaving only the general tissue reaction (Gentle and Tilston 1999). Studies on the neural activity in the medullary dorsal horn of monkeys suggest that attention-dependent changes in sensory discrimination and affective components of pain are mediated at the early stages of sensory processing (Hayes et al. 1981). If similar changes occur processing nociceptive information at the spinal level, it may also affect the activity of the peripheral nervous system (Gentle and Tilston 1999). Reduced pain perception and inflammation was demonstrated following attentional changes in the chicken (Gentle and Tilston 1999). However, more research is necessary before any clear conclusions can be drawn.

2.1.2 Control of Pain

Recognition of pain and anxiety in animals is critical for appropriate analgesic selection and pain relief. Furthermore, timely administration of analgesics is important as ongoing pain perception can have a negative effect on homeostasis and healing (Benson et al. 2000). Analgesia is the relief of pain without loss of consciousness and analgesics function by decreasing stimulation of the ascending spinal pathways or by activating the endogenous descending pain modulation pathways (Clyde 1994). When dealing with conditions in animals known to be painful in humans it is essential to treat

for pain (Flecknell 1988). Also if the procedure or injury involves tissue damage and/or the bird demonstrates changes in posture (guarding), temperament (aggressive or passive), or normal behaviour (i.e. decline of feeding or activity) the veterinarian should assume that the bird is in pain (Jenkins 1993). Controlling pain involves pharmacologic, physical, environmental and behavioural management (Wright et al. 1985). The contribution of proper care and nonchemical methods of analgesia should not be overlooked in any pain management program. The removal of fear and anxiety can reduce muscle tension and central nervous system activity. Anxiolytics, tranquillizers and muscle relaxants can be used in addition to proper support or bandaging of the traumatized area. Appropriate environmental modification with appropriate choice and location of perches, bedding, food and water to make a patient more comfortable. A dry, warm, quiet, nonstressful environment is essential (Clyde and Paul-Murphy 1999).

Lack of adequate information on pain and analgesia make choosing an appropriate analgesic difficult. Recognizing the signs of pain in birds is complicated by confounding factors such as behavioural differences between acute and chronic pain, between domestic and wild animals, and between predator and prey species, and differences in response between individuals (Paul-Murphy and Ludders 2001). We expect these differences between cows and dogs but often lump all avian species into one category. Effective analgesia of the avian patient will be more successful if these differences are recognized. Research evaluating pain thresholds and changes in the thresholds after administration of analgesic agents is limited in birds (Paul-Murphy et al. 1999). In addition, there is almost no information available on pharmacokinetics and

pharmacodynamics of analgesics in avian species but pharmacological intervention should be used as it would be in mammals. In mammals, opioids and alpha2-agonists are usually chosen for acute, sharp pain, whereas, nonsteroidal anti-inflammatory drugs are often administered for inflammation and chronic pain (Wright et al. 1985).

2.1.2.1 Pre-emptive Analgesia

Injury can induce prolonged changes in CNS function that later influences responses to subsequent afferent inputs and contributes to post-operative pain (Katz et al. 1992). Nociceptive information that reaches the spinal cord can produce central sensitization. Studies in mammals show that pain-induced neural changes can be prevented by administration of analgesic agents before development of injury induces spinal hyperexcitability and pain related behaviours (Coderre et al. 1990; Katz et al. 1992). In addition, analgesics are less effective when administered after prolonged central excitability or pain behaviour has already been established. Pre-emptive analgesia blocks sensory nociceptive stimuli from onward transmission thus reducing overall pain experienced by the animal.

2.1.2.2 Balanced Anesthesia

Balanced anesthesia refers to the administration of several drugs to prevent excess physiologic derangements by any single drug during or after anesthesia (Concannon et al. 1995). However, most birds are usually anesthetized solely with an inhaled anesthetic, frequently isoflurane (Ludders and Matthews 1996). During isoflurane anesthesia, the CNS is depressed sufficiently to prevent the perception of pain (Pascoe 2000) but it is important to remember that isoflurane-anesthesia does not provide post-operative analgesia (Dohoo 1990; Pascoe 2000). In fact, all inhaled anesthetics can be hyperalgesic (antinociceptive) at very low concentrations (i.e., concentrations that would be obtained at some point during recovery from anesthesia) by enhancing C-fibre activity (Zhang et al. 2000). Patients may perceive noxious stimulation from their wounds to be more intense than if no anesthetic were present (Zhang et al. 2000). Birds are known for a violent recovery from inhalant anesthesia (Ludders and Matthews 1996). If hyperalgesia is also present in avian species when inhaled anesthetics are at low concentration, it is possible that some of this behaviour during recovery may be attributable to intense pain. Thus, provision of appropriate analgesia may help to improve recovery in avian species.

In chickens, μ- (morphine) and κ-opioid (U50488H) agonists decrease requirements for isoflurane in a dose-dependent manner (Concannon et al. 1995). Combining these opioids with isoflurane had little effect on heart rate and mean arterial pressure but has the potential for respiratory depression (Concannon et al. 1995).

Despite a good margin of cardiovascular stability in a variety of mammalian species, isoflurane at high concentrations can depress both the cardiovascular and respiratory systems in avian species, particularly debilitated birds (Ludders and Matthews 1996). Therefore a balanced approach to anesthetizing avian patients may minimize the adverse effects of any one single drug while providing analgesia. In addition, combining agents may maximize analgesia.

2.1.2.3 Opioid Analgesics

Opioids exert their actions by binding to specific membrane receptors that are distributed throughout central and peripheral nervous system structures involved in transmission, modulation and sensation of pain. The three main classes of opioid receptors are mu (μ), delta (δ), and kappa(κ). Although the μ-receptor is most commonly associated with pain relief, specific δ- and κ-agonists can also modulate pain at spinal and supraspinal sites (Kanjhan 1995). Opioids have been considered as analgesics in avian species with variable and conflicting results (Bardo and Hughes 1978; Concannon et al. 1995; Curro et al. 1994; Hughes 1990; Paul-Murphy et al. 1999). Clinical use of opioids has been hindered by lack of published information concerning possible differences in opioid actions between avian and mammalian species. In mammals, μ- and κ-opioid agonists are often used to provide analgesia and CNS depression during anesthesia which results in an overall reduction in concentration of volatile anesthetics required (Concannon et al. 1995). Side effects such as sedation and

respiratory depression can be readily reversed with naloxone or naltrexone but this also terminates analgesia (Pascoe 2000).

In the pigeon, the effect of μ- and κ-agonists appears to be similar to mammals (Concannon et al. 1995). Autoradiographic studies of the forebrain of pigeons shows a predominance of κ-receptors in comparison to mammals (Mansour et al. 1988) but both μ- and κ-agonists are capable of producing analgesia in this species (Concannon et al. 1995). Pigeons are able to discriminate between a IM injection of morphine (μ-agonist) and saline but they are unable to distinguish μ-like compounds from κ-like compounds. In comparison, mammals are able to distinguish μ-like compounds from κ-like compounds, perhaps suggesting that the discriminative effects of these two classes of drugs share a common mechanism of action in pigeons (Herling et al. 1980). Differences in response to opioid analgesics may be related to the proportion of subclasses of opioid receptors present in different species (Clyde and Paul-Murphy 1999) but more research is necessary to determine opioid function in the forebrain.

In chickens, initial studies using high doses (200 mg/kg) of morphine produced analgesia to a toe pinch test (Schneider 1961), however, more recent studies demonstrate morphine analgesia at much lower doses (5 to 30mg/kg) using alternative nociceptive tests (Bardo and Hughes 1978; Fan et al. 1981). Chickens can be trained to associate colour with the presence of analgesic agents in their food. Chickens (both healthy and lame) selected food with the highest dose of morphine when given food with 3 different doses of morphine (8.6, 49, and 430 mg/kg food). Chickens without lameness also showed an obvious preference for morphine, perhaps for its euphoric effect (Danbury et

al. 1997). In domestic fowl, morphine can produce either hypoalgesia or hyperalgesia during thermal and chemical nociceptive tests. Genetic factors play an important role in determining sensitivity to opioid analgesic effects (Martin 1984). The hyperalgesia is displayed in domestic fowl and is strain dependent, naloxone sensitive, (Hughes 1990; Hughes 1990; Hughes et al. 1992; Sufka et al. 1991) and is mediated primarily by μ-receptor activation at CNS loci (Hughes and Baker 1995; Sufka et al. 1991).

Buprenorphine is a partial agonist that binds readily to μ-receptors and has some κ-antagonist properties. Being a partial agonist, it does not induce the same degree of effect as a full agonist such as morphine . It is only effective for treating mild to moderate pain (Pascoe 2000). Buprenorphine has been reported to be clinically effective in birds (Jenkins 1993) but in African gray parrots, large doses produced no significant analgesic effect (Clyde and Paul-Murphy 1999). However, increasing the dose significantly may result in reduced analgesic effect (Pascoe 2000).

Butorphanol is a synthetic opioid agonist-antagonist used commonly in small and large animal anesthesia for premedication and analgesia. Butorphanol is a weak antagonist at the μ-receptor but a strong agonist at the κ-receptor (Plumb 1995). In mammals, butorphanol produces analgesia in a dose-dependent manner with less respiratory depressant effects compared to morphine. In parrots, the analgesic effect of butorphanol was assessed by measuring the isoflurane-sparing effect (reduction in minimum anesthetic concentration). The addition of butorphanol to isoflurane decreased the amount of isoflurane required during application of a painful stimulus to cockatoos by 25% and by 11% in African gray parrots, but in blue-fronted Amazon parrots, the

amount of isoflurane required did not change significantly. (Curro 1994; Curro et al. 1994). However, care should be taken when interpreting this kind of information as isoflurane-sparing can be accomplished through sedation rather than analgesia (Dohoo 1990). In parrots, butorphanol has minimal respiratory effects as it produces an increase in respiratory frequency with a decrease in tidal volume but without a significant change in minute volume (Curro et al. 1994). Another study was unable to demonstrate a reduction in the amount of halothane required to anesthetize turkeys during surgery with the addition of butorphanol. However, birds treated with butorphanol had fewer responses to noxious stimuli than did controls (Reim and Middleton 1995).

2.1.2.4 Nonnarcotic Analgesics

2.1.2.4.1 Anti-inflammatory Drugs

2.1.2.4.1.1 Steroidal Anti-inflammatory drugs (Corticosteroids)

Corticosteroids may reduce pain by suppressing response to chemical, thermal, traumatic, or inflammatory injury through reduction in fibroblast proliferation, macrophage response to migration inhibition factor, sensitization of lymphocytes, and response to mediators of inflammation (Plumb 1995). The combination of long-acting anesthetic agents (i.e. bupivacaine) and corticosteroids has been shown to reduce postoperative discomfort in humans (Glasser et al. 1993). Bethamethasone is a powerful steroidal anti-inflammatory drug that reduces pain associated with degenerative hip disorders in adult male turkeys (Duncan et al. 1991). It has also been used to decrease

inflammation in uric acid induced hock-joint pain in chickens (Hocking et al. 1999).

Corticosteroids can alter response to endogenous or parenterally administered opioids. In the rat, administration of a potent synthetic corticosteroid, such as dexamethasone, can reduce the antinociception induced by μ-agonists while potentiating κ- agonists (Pieretti et al. 1994). The administration of corticosteriods may, therefore, potentially reverse stress-induced analgesia acting at the μ-receptor and caution should be used with stressed patients. Polydypsia, polyphagia and polyuria may be seen at inflammatory doses but adverse effects are relatively uncommon in mammals (Plumb 1995). However, risk of possible immunosuppression and other potential complications make nonsteroidal anti-inflammatory drugs preferable in many situations (Clyde and Paul-Murphy 1999).

2.1.2.4.1.2 Non-steroidal Anti-inflammatory drugs (NSAIDs)

Prostaglandins (PGs) are important local mediators of inflammation and pain, and are also known to lower activation threshold to thermal, mechanical and chemical stimulation. Nonsteroidal anti-inflammatory drugs control pain by inhibiting the cyclooxygenase enzyme which prevents the production of PGs. Drugs that inhibit the prostaglandin biosynthesis in mammals produce analgesia by decreasing inflammation at the site of injury and also through central nervous system effects (Mathews 2000). Prostaglandins are involved in the modulation of avian pain responses and the physiológical mechanisms involving PGs are similar to that described in mammalian models (Nicol et al. 1992).

Prostaglandin synthesis is mediated by one of two isoforms of cyclooxygenase enzymes: cyclooxygenase-1 (COX-1) and cyclooxygenage-2 (COX-2). The COX-1 enzyme is constitutive (part of the normal enzyme complement of a cell) and present at relatively constant concentrations. In comparison, COX-2 is inducible and concentrations increase in response to a stimulus. Cyclo-oxygenase-1 produces prostaglandins that have a cytoprotective function in tissues such as the gastric mucosa, kidneys, reproductive tract and central nervous system. Similarly, the production of thromboxane in platelets is a COX-1 mediated process. Until recently, NSAIDs were believed to have exerted their therapeutically beneficial effects primarily by inhibiting COX-2, while drugs which inhibit COX-1 was responsible for some of the toxic side effects such as gastric ulceration, renal papillary damage, and extended clotting time (Nolan 2000). Consequently, there has been a shift in focus to drugs that inhibit COX-2 (Livingston 2000). Although COX-2 selective drugs do not induce gastric ulceration, COX-2 is expressed in low amounts in the healthy stomach and appears to play an important role in promoting ulcer healing. It also appears that COX-1 contributes to the inflammatory process and COX-2 selective inhibitors may not be as efficacious as mixed inhibitors in their anti-inflammatory actions. Both COX-1 and COX-2 are constitutively expressed in the CNS and their relative expression varies depending on species (Nolan 2000). Renal perfusion in hypovolemia is supported by prostaglandins, but studies indicate that COX-1 and COX-2 are present in kidneys of some species (Livingston 2000). It is clear that more research is necessary to determine the importance of these isoenzymes.

While inhibition of COX-2 activity is the most likely mechanism of action for NSAID-mediated analgesia there is increasing evidence that NSAIDs have a central mechanism that augments the peripheral mechanism. Several studies in animals and humans have shown that NSAIDs may also be reversed by naloxone, possibly demonstrating a central opioid mechanism of action of antinociception (Cashman 1996). It is likely that both COX-1 and COX-2 are important in anti-nociception but more research is necessary to distinguish these effects. Flunixin, ketoprofen and carprofen have COX-1 and COX-2 actions (Swan et al. 1995).

Some studies have investigated specific NSAIDs using avian models. Pharmacokinetic studies with broiler chickens indicate that peak plasma levels of carprofen are reached between 1 and 2 hours after a subcutaneous dose, and in preliminary tests of pain thresholds, a dose of 1 mg/kg of carprofen raised pressure thresholds for at least 90 minutes after a SQ injection (McGeown et al. 1999). However, plasma levels of NSAIDs likely do not reflect physiological or pharmacological activity (Owens et al. 1995) as NSAIDs are weak acids,(Landoni et al. 1995) highly protein bound, and tend to accumulate in areas of inflammation (Lees et al. 1987). Therefore, plasma thromboxane B_2 levels may better estimate duration of drug action because NSAIDs may produce effects longer than would be predicted from their actual plasma levels. In mallard ducks, flunixin (5mg/kg) and ketoprofen (5 mg/kg) suppressed thromboxane B_2 levels for up to 12 hours possibly suggesting that their physiologic action may be that long (see Chapter 3, Machin et al. 2001) but further studies are necessary. Pharmacokinetic data cannot be extrapolated between species (Nolan 2000).

Lame chickens preferentially select food with carprofen at 3 doses (3.4, 34.3, and 343.0 mg/kg food) compared to food without analgesics (Danbury et al. 1997). However, healthy chickens showed an aversion to the highest dose which may reflect an aversive taste or the occurrence of side effects such as nausea (Flower et al. 1980). In another study, carprofen increased the speed and walking ability of rapidly growing broiler chickens with chronic lameness (McGeown et al. 1999). Other NSAIDs have been used in avian species with some success although renal toxicity and gastrointestinal effects have been noted in some clinical cases. In parrots, flunixin did not produce an isoflurane sparing effect at 4mg/kg IM (Curro 1994), possibly suggesting that higher doses are required in this species. Recommended doses range from 1 to 10 mg/kg (Ritchie and Harrison 1994), however, in birds there are no experimental data available to confirm analgesia at low doses. Chickens given phenylbutazone applied topically to the beak were able to maintain their pre-trimming feed intake levels over the first 24 hours after the procedure and this was higher than in untreated birds (Glatz et al. 1992).

2.1.2.4.2 Alpha2-Adrenergic Agonists

As in mammals, avian sensitivity to noxious stimuli is susceptible to adrenergic modulation. Alpha$_2$-agonist activation can produce sedation, anxiolysis, analgesia and reduction in minimum alveolar concentration of inhalant anesthetics (Maze and Tranquilli 1991). Alpha$_2$-agonists (such as xylazine and medetomedine) alone in birds produces muscle tremors, respiratory depression, salivation and movement in response to

noise (Redig 1982; Samour et al. 1984). Therefore, they are often combined with ketamine. Disadvantages include hypertension following IV bolus injections, hypotension, bradycardia with partial A-V block, (Redig 1982; Samour et al. 1984) dose dependent hypothermia by decreasing thermogenesis (Livingston et al. 1984), increased postoperative fluid requirements (Milne 1991), sedation, and respiratory depression. Although inclusion of alpha$_2$-agonists can be useful in premedication for balanced anesthesia during painful procedures, post operative administration of alpha$_2$-agonists is not usually performed. Atipamezole, is a highly potent, specific, competitive alpha$_2$-antagonist of centrally and peripherally located α_2-adrenoceptors that will quickly relieve any unwanted side effects but administration also reverses analgesia (Virtanen 1989).

2.1.2.4.3 Ketamine

Ketamine is a dissociative anesthetic and is an N-methyl-D-aspartate (NMDA) glutamate receptor antagonist (Lamont et al. 2000; Siddell and Cousins 1995). Ketamine is often combined with sedatives such as alpha$_2$-agonists and benzodiazepines for premedication or general anesthesia for minor procedures. At low doses, ketamine can enhance analgesia by preventing NMDA receptor-mediated sensitization in the central nervous system. Therefore, ketamine is useful for pre-emptive analgesia in major surgeries and also for post-operative analgesia as it may abolish hypersensitivity once it is established (Lamont et al. 2000). Although ketamine prevents sharp superficial pain

effectively, visceral, dull pain is not controlled. Therefore, analgesia produced by ketamine alone is not adequate for laparotomies or orthopedic surgery (White and Holmes 1976).

2.1.2.4.4 Local Anesthetics

Local anesthetics (i.e. lidocaine, bupivacaine) function by blocking ion channels thereby preventing generation and conduction of pain impulses (Plumb 1995). Local anesthesia before tissue trauma can significantly reduce postoperative pain because it prevents nociceptor sensitization and therefore avoids central changes that are secondary to activation of pain pathways (Clyde and Paul-Murphy 1999). Local nerve blockade before nerve transection in amputation can decrease the prevalence of "phantom limb" pain in humans (Coderre et al. 1993). Although local anesthesia is sufficient for pain relief it does not reduce stress that may be induced by physical restraint and handling of an awake bird (Ludders and Matthews 1996). Sedation or general anesthesia should also be considered during stressful or prolonged procedures.

Birds may be more sensitive to the toxic effects of local anesthetics than mammals, as lower doses in birds (2.7 - 3.3 mg/kg) produce toxic effects (Hocking et al. 1997) compared with higher doses (3.5 -4.5 mg/kg) in dogs (Skarda 1996). It is recommenced that lidocaine dose not exceed 4 mg/kg in birds as seizures and cardiac arrest can be produced with overdose (Ludders and Matthews 1996). Chickens receiving bupivacaine (2.7 - 3.3 mg/kg) showed signs of toxicity (recumbency with outstretched

legs, drowsiness) and distress immediately after injection (Hocking et al. 1997). Adverse cardiac effects only generally occur at high plasma concentrations and are associated with prolonged PR and QRS intervals and shortened QT intervals. Other adverse side effects of local anesthesia can also depression, drowsiness, ataxia, nystagmus, muscle tremors, and possibly hypotension (Plumb 1995).

The length of action of local anesthetics in avian species is unknown. In mammals, lidocaine is a shorter acting (60-120 minutes) than bupivacaine (240-360 minutes) (Lemke and Dawson 2000). In domestic fowl, bupivacaine produces effective analgesia in two pain models. Chickens that had topical bupivacaine applied to the beak stump after amputation were able to maintain their pre-trimming feed intake levels over the first four hours after the procedure (Glatz et al. 1992). Intra-articular injection of sodium urate produces acute synovitis with inflammatory changes such as swelling, increased joint temperature, and sensitization of the joint capsule receptors lasting at least 3 hours (Gentle 1997; Hocking et al. 1997). In this model, intra-articular bupivacaine increased feeding, pecking, and standing behaviours while the proportion of resting declined. Birds treated with bupivacaine (2 mg/kg) were indistinguishable from animals in the untreated control group (Hocking et al. 1997).

Pain perception in birds is likely analogous to mammals and invasive and painful procedures should always be accompanied by appropriate analgesia and anesthesia. When choosing an analgesic for an avian patient, the practitioner should consider the level of pain and treat appropriately as they would in mammalian species. Pharmacological intervention is important but physical, environmental and behavioural management should not be overlooked (Clyde and Paul-Murphy 1999). Although avian pain

management is in its infancy, research and clinical studies demonstrate the benefit for the use of opioids, steroidal and nonsteroidal anti-inflammatory drugs, as well as other analgesics such as α_2-agonists, ketamine and local anesthetics. Assessment of analgesic efficacy is extremely important as the dosage and choice of analgesic may vary widely between species. The information given in this chapter is meant to be used as a guide for treatment of pain in avian species rather than recommendation. Clearly there is a need for further clinical investigations and both successes and failures should be reported in the veterinary literature to expand the limited information available.

3. EXPERIMENTAL EVALUATION OF THE PHARMACODYNAMICS OF FLUNIXIN AND KETOPROFEN IN MALLARD DUCKS

3.1 Abstract

Flunixin (FLX) and ketoprofen (KET) are potent non-steroidal anti-inflammatory drugs (NSAIDs) used to alleviate pain and decrease inflammation. These drugs block access of arachidonic acid to its binding site on the cyclo-oxygenase enzyme thus preventing conversion to thromboxane B_2 (TBX). Consequently, TBX may be used to estimate the duration of NSAID action. Sixteen adult mallard ducks were assigned randomly to 3 treatment groups: control (n = 4), FLX (5 mg/kg) (n = 6) or KET (5 mg/kg) (n = 6). Blood samples were taken at 1 h prior to and just before (0 h) injection, and 0.25, 0.5, 1, 2, 4, 6, 12, 24, 36, and 48 h after injection. Samples were analysed for corticosterone and TBX. Feces were tested for the presence of hemoglobin to detect gastrointestinal bleeding and ducks were euthanised for complete necropsy at the end of the study. Samples of muscle, kidney, liver, proventriculus and intestine were taken for histological analysis. Thromboxane was suppressed significantly in all birds following administration of either FLX or KET for 4 h compared to baseline samples (-1 and 0 h) but decreased for approximately 12 h. In comparison, TBX in the control group gradually declined from the start of the experiment. None of the ducks had evidence of

gastrointestinal bleeding but FLX had muscle necrosis present at injection sites. FLX and KET likely exert pharmacological effects for at least 12 h. Although the degree of TBX inhibition has not been correlated accurately with the degree of analgesia or anti-inflammatory effects, it is likely that these effects are present during this time. This work suggests that FLX and KET can potentially be used as anti-inflammatory agents in waterfowl. However, because muscular necrosis was produced at the injection site, FLX is not recommended in ducks.

4.2 Introduction

Flunixin (FLX) and ketoprofen (KET) are potent non-steroidal anti-inflammatory drugs (NSAIDs) used therapeutically in human and veterinary medicine to alleviate pain and decrease inflammation (Kantor 1986; Swan et al. 1995; Watson et al. 1996). In avian species, FLX has been recommended for pain relief (Ritchie and Harrison 1994) but use of KET has not been reported and the pharmacokinetics and pharmacodynamics of these NSAIDS in birds are unknown. In dogs and cats, both FLX and KET provide appropriate analgesia for post-surgical pain with a half life of 3 to 4 h (Swan et al. 1995) and 1 to 3 h, respectively (Lees and Taylor 1991; Soraci et al. 2000; Swan et al. 1995). Anti-inflammatory, analgesic, antipyretic, and antithrombotic actions of NSAIDs are brought about by inhibition of prostaglandin production. More specifically, NSAIDs are competitive antagonists to the enzyme cyclo-oxygenase thus preventing conversion of arachadonic acid to prostaglandin H_2 and thromboxane A_2. Prostaglandin H_2 is metabolized to a range of other prostaglandins (PGE_2, PGD_2, PGF_2 and PGI_2) and

thromboxane A_2 is subsequently degraded chemically to thromboxane B_2 (TBX) (Vane 1971). Prostaglandin synthesis is mediated by one of two isoforms of cyclooxygenase enzymes: cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). The COX-1 enzyme is constitutive (part of the normal enzyme complement of a cell) and present at relatively constant concentrations. In comparison, COX-2 is inducible and concentrations increase in response to a stimulus. Cyclo-oxygenase-1 produces prostaglandins that have a cytoprotective function in tissues such as the gastric mucosa, kidneys, reproductive tract and central nervous system. Similarly, the production of thromboxane in platelets is a COX-1 mediated process (Livingston 2000; Nolan 2000).

Until recently, NSAIDs were believed to have exerted their therapeutically beneficial effects primarily by inhibiting COX-2, while drugs which inhibit COX-1 were responsible for the toxic side effects such as gastric ulceration, renal papillary damage, and extended clotting time. Consequently, there has been a shift in focus to drugs that inhibit COX-2 (Livingston 2000). However, while highly COX-2 selective drugs do not induce gastric ulceration, COX-2 is expressed in low amounts in the healthy stomach and appears to play an important role in promoting ulcer healing. It also appears that COX-1 contributes to the inflammatory process and COX-2 selective inhibitors may not be as efficacious as mixed inhibitors in their anti-inflammatory actions. Both COX-1 and COX-2 are constitutively expressed in the CNS and their relative expression varies depending on species (Livingston 2000).

NSAIDs may produce effects longer than would be predicted from their actual plasma levels (Higgins and Lees 1984). Plasma levels of NSAIDs do not reflect

physiological or pharmacological activity (Owens et al. 1995) as NSAIDs are weak acids (Landoni et al. 1995), are highly protein bound, and tend to accumulate in areas of inflammation (Lees et al. 1987). Greater drug levels in sites of inflammation have also been attributed to increased blood flow, increased vascular permeability and increased protein passage to these sites (Higgins and Lees 1984). Studies have shown a strong correlation between the actions of NSAIDS on thromboxane B_2 in the plasma and PGE_2 in the exudate for both ketoprofen and flunixin (Landoni et al. 1995; Toutain et al. 1994). Since the inhibition of PGE_2 in inflammatory exudate is a significant component of the clinical effect of NSAIDS, it is not unreasonable to attempt to equate TBX concentration with clinical response. The same dose of ketoprofen in horses used in pharmacokinetic/pharmacodynamic studies (Landoni et al. 1995), produces clinical effects, such as the suppression of lameness, for the same time period as the effect on plasma TBX (Owens and Kamerling 1995; Owens et al. 1995). However, not all clinical effects correlate well with eicosinoid suppression, and central effects on analgesia may be significant (Dirig and Yaksh 1997). Clinical effects may be affected by other factors, such as different COX1 : COX2 specificity since the TBX synthesis is related to COX 1 while the inflammatory response is related to COX2 (Livingston 2000). But as neither FLX nor KET show particular specificity for either COX isoenzyme (Livingston 2000), plasma TBX is probably the best indicator available to estimate duration of drug action.

In avian species, there is some evidence that NSAIDs may not provide effective analgesia at low doses. Flunixin did not produce an isoflurane sparing effect in parrots at 4 mg/kg i.m. (Curro 1994) and 0.5 to 2.0 mg/kg i.m. ketoprofen did not appear to

provide analgesia after skin incision in mallard ducks (Machin 1998), possibly suggesting that higher doses are required for analgesia. Therefore, a dose of 5mg/kg was chosen in this study because it is midpoint in the recommended dose range of 1 to 10 mg/kg for flunixin in avian species (Ritchie and Harrison 1994).

Stress may also play an important role in TBX suppression. Glucocorticoids, such as corticosterone, can inhibit prostaglandin production resulting in decreased TBX production (Tomchek et al. 1991). As the mallard ducks used in this study were wild-strain, corticosterone levels were measured to determine if stress played a role in TBX suppression in treated and control groups. The primary purpose of this study was to investigate the pharmacodynamics of two NSAIDs, FLX and KET, in mallard ducks by measuring serum TBX.

3.3 Materials and Methods

Sixteen healthy adult mallard ducks (*Anas platyrhynchos*; eight male, eight female), F2 progeny of wild-strain adults, weighing 1055 ± 114 g (mean ± SD) and 943 ± 85 g, respectively, were used for the study. Ducks were determined to be healthy on the basis of physical examination and complete blood count (CBC). All ducks were maintained individually in cages (75 cm^3) with a 20 x 40 cm pool and fed duck and goose grower diet *ad libitum*. The cages were positioned so that ducks in the cages could see the duck in the neighbouring cage and other ducks free in the room.

Ducks were assigned randomly to 3 treatment groups: (a) control (n = 4), (b) flunixin (FLX, 5 mg/kg, n = 6, Banamine, Schering-Plough Animal Health, Point-Claire, Québec, Canada) or (c) ketoprofen (KET, 5 mg/kg, n = 6, Anafen, Rhône Mérieux Inc. Athens, Georgia). Each of the treatment groups had equal number of male and female ducks. Two ducks in the control group had a jugular catheter placed during isoflurane (IsoFlo, Abbot Laboratories Limited, Saint-Laurent, Québec, Canada) anesthesia and subcutaneous bupivacaine (2 mg/kg, Marcaine, Sanofi Winthrop, Chatham, Ontario, Canada) for post-operative analgesia. As catheterization can increase circulating thromboxane levels (Bailey et al. 1983), two ducks were anesthetized with isoflurane but received neither a jugular catheter nor bupivicaine. Ducks in the latter group had blood samples taken from either the jugular or brachial veins and were not given heparin. Sampling from a single vein throughout the experiment was not always possible because of hematoma formation.

All ducks in the FLX and KET groups had a jugular catheter placed during isoflurane anesthesia but bupivicaine was not given. Anesthesia was induced using 5% isoflurane in O_2 by mask delivery. When the birds were anesthetized, they were intubated with a 3.0 mm non-cuffed endotracheal tube and maintained on 2.0 to 3.0 % isoflurane at a flow rate of 1.0 L/min through a non-rebreathing system for the surgical procedure. The right jugular vein was isolated surgically in ducks that received catheters and a 20 gauge, 5.0 cm catheter (approximate volume, including hub, 0.15 mL) was inserted for the purpose of repeated blood sampling.

Blood samples of 0.3 mL were drawn into heparinized syringes (1000 units/mL) at -1 h (60 minutes prior to catheter placement) and 0 h (after induction isoflurane) from

the brachial vein. Immediately after induction and collection of the 0 h sample, either FLX or KET at 5 mg/kg was administered i.m. into the left pectoral muscle. Equal volumes (1.2 mL) were given by diluting the NSAID with sterile saline. Ducks in the control group received an equal volume of saline i.m. in the left pectoral muscle. Blood samples of 0.5 mL were taken at 15 minutes, 30 minutes, and 1, 2, 4, 6, 12, 24, 36, and 48 h. Time 0 and 15 minute samples were taken during anesthesia. During sampling, 0.1 mL of heparinized saline was injected into the catheter and 0.5 mL of blood was withdrawn before collecting the blood sample to prevent dilutional error. The blood sample was withdrawn and placed in an integrated plasma separation tube with lithium heparin (Sherwood Davis and Geck Medical, St Louis, MO). To maintain blood volume the initial 0.5 mL of blood was replaced and catheter patency was maintained by flushing with 0.5 mL heparinized saline after each sample. Blood samples were centrifuged at 2000 x g for 10 minutes and samples were then frozen at -20 °C until analysis.

Duplicate samples from each time point from individual ducks were analysed for TBX (Craig-Schmidt et al. 1986) using a TBX Enzyme Immunoassay Kit (Cayman Chemical Company, Ann Arbor, MI) and for corticosterone using a Double Antibody Rat Corticosterone Kit (Sorenson et al. 1997) (ICN Pharmaceuticals Inc., Orangeburg, NY, 10962). Analysis was done according to the instructions provided by the manufacturers. For TBX, the manufacturer reports 100 % specificity, and cross reaction of 8.2% with 2,3-dinor-thromboxane B2, and < 5 % cross reaction with prostaglandins D_2, E_2, $F_{1\alpha}$, $F_{2\alpha}$, 6-keto prostaglandin $F_{1\alpha}$, 2,3-dinor-6-keto prostaglandin $F_{1\alpha}$, 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$, 11-dehydro-thromboxane B_2, and leukotriene B_4

(Cayman Chemical Company, Ann Arbor, MI). Coefficients of variation for this study was 5.44 ± 4.71 (mean ± standard deviation) for TBX and 460.6 ± 9.3 for corticosterone.

Feces were tested at 0, 24 and 48 h for the presence of hemoglobin using Haematest tablets (Miles Canada Inc., Etobicoke, Canada) to detect gastrointestinal bleeding. Ducks were euthanised for complete necropsy at the end of the study. Histologic examination was performed on five ducks from each of the treated groups (FLX and KET). Samples of muscle (injection site), kidney, liver, proventriculus and intestine were fixed and stored in 10% neutral buffered formalin before being embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. To characterize intra-hepatocyte pigmented material, selected sections of liver were also stained with Prussian blue (Luna 1968). Sections were evaluated for histologic changes by an observer blind to the treatments.

Data are reported as mean ± standard error. Wilcoxon signed-rank tests were used to determine if there were significant differences between time 0 and -1 h before combining results. Plasma corticosterone and TBX suppression data were analysed using a repeated measures analysis of variance (ANOVA) for the average value derived from duplicate samples. In addition, TBX suppression results from both sexes and control groups were examined using repeated measures ANOVAs to determine if there were significant differences between groups before combining results. Thromboxane B_2 data were log transformed to avoid violating the normality assumption of the ANOVA. Where significant differences occurred, a post-hoc Fisher's least-significant-difference pairwise comparison was used to compare results with corticosterone time -1 h and TBX

baseline (average of time 0 and -1 h). Results were considered significant when P ≤ 0.05.

3.4 Results

One apparently healthy male duck given FLX was removed from the study due to the presence of systemic mycobacteriosis at necropsy. As there was no difference in TBX suppression between males and females or between both control groups, results from both sexes and control groups were combined. Thromboxane was suppressed in all birds following administration of either FLX or KET (Figures 3.1 and 3.2). With regard to the overall profile, there was no significant difference between drugs ($F = 1.05$, $df = 10$, $P = 0.392$) but TBX suppression by KET did not appear to be as long lasting as with FLX (Figure 3.2). Significant suppression, for both NSAIDs, occurred by 15 minutes and maximal TBX suppression was achieved by 30 minutes. Significant suppression, for both drugs, lasted until 4 h post-injection ($F = 30.3$, $df = 10$, $p = 0.0001$, Figure 3.1) but TBX remained suppressed at 6 and 12 h (Figures 3.1 and 3.2). Suppression was followed by an increase in TBX levels which were above baseline (not significant) in the KET group (Figure 3.1).

Ducks in the control group had a gradual decline in TBX from the start of the experiment (time 0 and -1 h) to 48 h. The decline was not related to any obvious circadian rhythm, the highest mean values (4299 ± 1419 pg/mL) were recorded at baseline (times -1 and 0 h) and the lowest value (570 ± 238 pg/mL) occurred at 24 h. At

36 (1033 ± 394 pg/mL) and 48 h (931 ± 265 pg/mL), TBX values were marginally higher than at 24 h but not as high as at baseline (Figure 3.1).

Corticosterone levels rose significantly from the initial sample (-1 h) in all groups (F_{FLX} = 4.11, df = 11, P = <0.001, F_{KET} = 5.175, df = 11, P = <0.001, $F_{CONTROL}$ = 9.224, df = 11, P = <0.001,). Levels were significantly different from the first sample taken (time -1 h) from 0 to 4 h in the control and KET groups and at time 0, 30 min, and 1 h in the FLX group. Although not significant, corticosterone levels were higher at 15 min and 2 h compared to -1 h in the FLX group (Figure 3.3). Sample size was reduced for corticosterone as there was insufficient plasma available for analysis for 22 of 144 samples.

None of the ducks had any evidence of gastrointestinal bleeding as indicated by the lack of hemoglobin in the feces. The most significant histologic finding was the presence of lesions of focally extensive muscular necrosis at the injection site of all ducks that received FLX. These areas of necrosis covered approximately 1-2 cm^3, and were characterized by coalescing zones of severe architectural disorganization of the muscular fibers. The affected myocytes were either shrunken or swollen, and had a hyper-acidophilic, fragmented, and coagulated cytoplasm. Partial to total mineralization of the degenerate sarcoplasm was often present. These necrotic myocytes were usually surrounded by a moderately abundant population of inflammatory cells mainly composed of macrophages and multinucleated cells. Moderately extensive fibrosis of the affected areas was seen in two of the five cases. The only other significant histologic finding was by the presence of small to moderate quantities of iron pigments (hemosiderin) in the hepatocytes of all but one duck.

3.5 Discussion

Compared to baseline, greater than 82 percent TBX suppression was achieved by 15 minutes, indicating that absorption and distribution of both NSAIDs was very rapid. Thromboxane B_2 was suppressed significantly in all ducks that received KET or FLX for 4 h and non-significant suppression lasted up to 12 h. Similar studies in other species show that duration of TBX suppression is variable and dose-dependent (Semrad et al. 1985). Inhibition of plasma TBX by FLX has been reported in horses ((Landoni and Lees 1995; Lees et al. 1987; Toutain et al. 1994), calves (Landoni et al. 1995; Landoni et al. 1995), dogs (McKellar et al. 1989), and cats (Lees and Taylor 1991). Significant blockade of TBX production with i.v. FLX in horses may be as short as 4 h (Soma et al. 1992) or as long as 24 h (Landoni and Lees 1995; Lees et al. 1987), with levels returning to normal by 12 and 24 h, respectively (Landoni and Lees 1995; Lees et al. 1987; Soma et al. 1992). In the cat, oral FLX produced significant suppression lasting for less than 7 h (Lees and Taylor 1991)) whereas in dogs it resulted in suppression for at least 12 h (McKellar et al. 1989). In addition, greatest variation in TBX suppression between dogs occurred at lower doses (McKellar et al. 1989).

For KET, TBX inhibition has been reported in horses (Landoni and Lees 1996), calves (Landoni et al. 1995; Landoni et al. 1995; Landoni and Lees 1995), sheep and rabbits (Suesa et al. 1993). In calves (Landoni et al. 1995) and horses (Landoni and Lees 1995), TBX synthesis was significantly inhibited after i.v. administration of KET for 12 h and nonsignificant suppression for 24h. Similarly, in sheep significant suppression lasted 8 h (Landoni et al. 1999).

A nonsignificant increase in TBX production was seen after suppression in this study. This is similar to that reported in other studies following administration of FLX and phenylbutazone in healthy horses but cause is unknown (Hardee and Moore 1986; Lees et al. 1987; Lees and Higgins 1985; Semrad et al. 1985). This increase in TBX production may be explained by an increase in circulating thrombocytes, an increase in cyclo-oxygenase or thromboxane sythestase in response to suppression, an increase in phospholipase activity releasing more arachidonic acid from the cell membranes, or a build up of arachidonic acid in membranes during the period of suppression (Hardee and Moore 1986). Alternatively, in response to low TBX, there may be a release of new thrombocytes which have a higher capacity to produce TBX. (Lees and Higgins 1985, Lees et al. 1987).

Stress from confinement, isolation, and multiple blood sampling resulted in elevated circulating corticosterone levels. These levels rose dramatically in all groups after the first sample and remained significantly high during frequent sampling (0 to 4 h). However, power to detect significant differences was low due to reduced sample size at some time periods. One study in pigeons demonstrated that plasma corticosterone concentrations were significantly higher in birds sampled at 4 h intervals compared with 28 h intervals (Westerhof et al. 1994). Another study in geese demonstrated that even minor routine handling procedures produced a marked stress response with significant elevations of corticosterone and catecholamines, this response was demonstrated even though birds appeared normal and had been habituated to the procedure for weeks before the study (Le Maho et al. 1992). As corticosterone levels were increased in all groups,

including control, the elevated corticosteroid levels were considered a response to stress rather than drug administration.

Although ducks in the control groups were divided into 2 sub-groups (with and without a catheter), all ducks showed a similar pattern of TBX decline over 48 h. No significant difference was detected between the groups, nor was there any obvious disparity between either sex or subgroup. Despite the differences in treatments, control birds all responded in a similar manner which led to the combination of the control ducks results for comparison with the treated groups.

The gradual decline in corticosterone, has not been reported in similar studies. Glucocorticoids, such as corticosterone, have anti-inflammatory effects mediated through inhibition of prostaglandin production (Clarke 1991). Glucocorticoids evoke lipocortin generation by tissue cells (Blackwell et al. 1980) which can result in platelet phospholipase inhibition (Aarsman et al. 1987), thus, decreasing TBX production (Breazile 1987). As glucocorticoids have been postulated to inhibit cyclooxygenase activity (Breazile 1987), it is possible to hypothesize that when corticosterone was high it stimulated production of lipocortin which resulted in TBX decline over time. In comparison, the gradual decline in TBX was not demonstrated in the FLX and KET groups. I hypothesize that when plasma TBX reaches very low levels, as seen with the thromboxane suppression by FLX and KET, there may be a mechanism for production of TBX which could override any stimulus to suppress its production described above. However, this has not been tested.

Administration of NSAIDs can produce a number of adverse effects, the most common in mammals being gastrointestinal irritation and ulceration (Kore 1990). The negative fecal occult blood tests suggests that a single pre-emptive dose of the NSAIDs at the dose given in this study did not produce significant gastrointestinal bleeding.

Lesions, similar to the ones seen in the pectoral musculature of the mallard ducks, at the injection site that received FLX have also been observed in northern bobwhite (Klein et al. 1994). One study in cattle (Pyorala et al. 1999), examined behavioural and clinical response (pain, erythema, edema) at injection site, and serum creatine kinase (CK) levels in response to NSAID injection. Flunixin produced greater adverse clinical responses and a greater magnitude of the CK rise in comparison to KET (Pyorala et al. 1999). Flunixin preparations contain propylene glycol which is known to cause lesions when injected i.m.(Svendsen et al. 1979) which may explain the muscle damage. The muscle damage caused by FLX in cattle was of greater magnitude than any of the other three NSAIDs tested in the study (Pyorala et al. 1999). Nephrotoxicity has been reported with the use of FLX in northern bobwhite (Klein et al. 1994) and in different species of mammals (Kore 1990). Anecdotal reports also suggest that repetitive use of FLX can cause lesions of renal gout in different species of birds (Klein et al. 1994). In the present study, the single administration of KET or FLX at 5 mg/kg did not produce any histologic changes to the kidney.

Mild to moderate quantities of iron pigments (hemosiderin) were found in the hepatocytes of all but one duck. Pigments of hemosiderin have been described in the hepatocytes of clinically healthy mallard ducks (Cork et al. 1995). In this study these

pigments were present in ducks from both treatment groups. Accordingly, the hepatic hemosiderosis observed in this study is not believed to be associated with either of the drugs.

The time course of platelet cyclo-oxygenase suppression was measured as an indicator of the drug's action. However, it is unknown if TBX inhibition can be correlated with the degree of analgesia or anti-inflammatory effects provided by NSAIDs for a number of reasons. Elevated drug concentrations are more persistent in damaged tissues than in plasma and there are likely to be different cyclo-oxygenase iso-enzymes in different tissues (Taylor et al. 1994). Cyclo-oxygenase inhibition is a major mechanism of action of NSAIDs but other biochemical and molecular actions have been described which can contribute to the biological actions of these drugs (Landoni et al. 1996). As TBX synthesis is related to COX 1 while the inflammatory response is related to COX2, different COX1 : COX2 NSAID specificity may result in dissimilar clinical effects (Livingston 2000). In addition, NSAIDs exert significant antinociceptive effects at the spinal level (Bustamante et al. 1997) which cannot be estimated through measurement of peripheral TBX. However, as FLX and KET suppress production of TBX for about 12 h, it is possible that some anti-inflammatory and analgesic effects are present during this time. Further studies are required to determine the effectiveness of NSAID administration for analgesia in birds.

In conclusion, FLX and KET may exert pharmacological effects for at least 12 h. Administration of NSAIDs may benefit waterfowl research by providing post operative analgesia; however, because of the extensive muscular necrosis associated with the i.m. injection of FLX, I do not recommend the use of this drug in ducks. Even though single

administration of FLX and KET did not seem to be associated with renal changes, the safety of repetitive use of these drugs on renal function in ducks still needs to be determined.

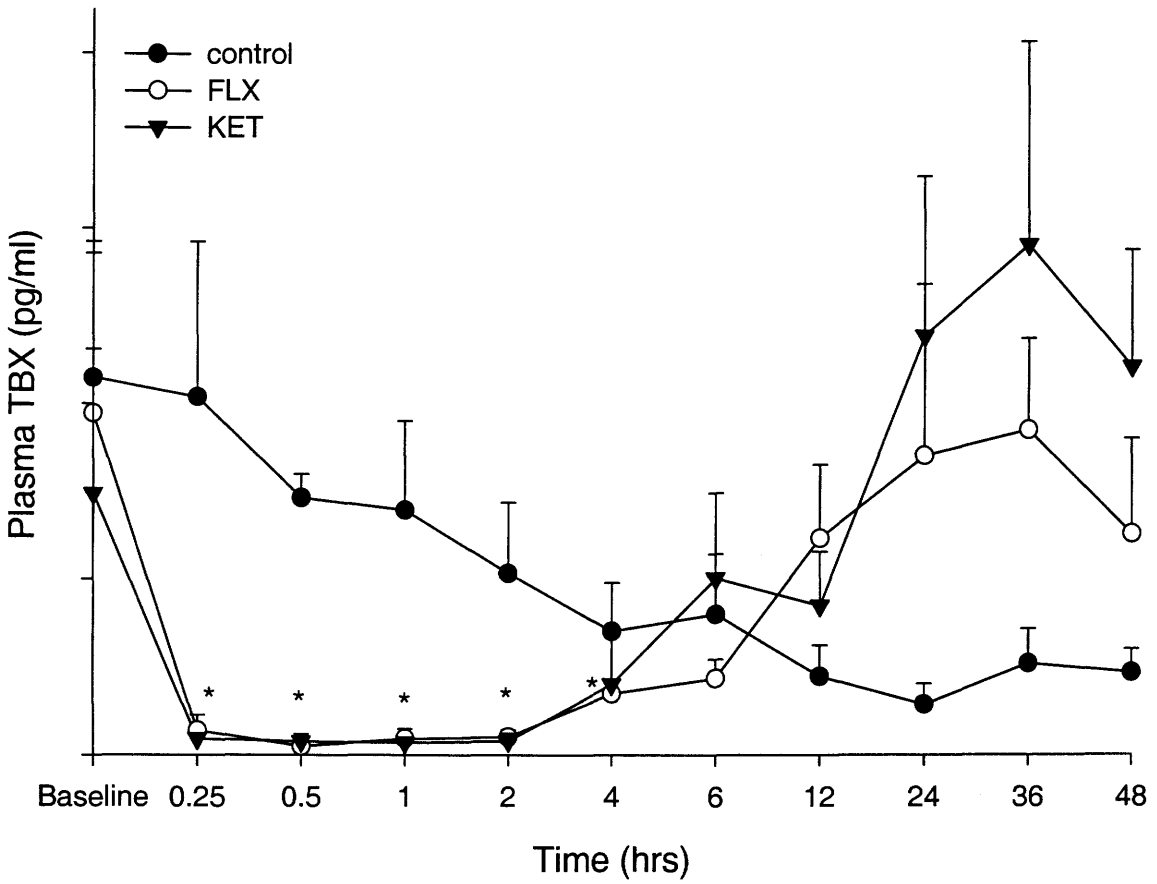

Figure 3.1. Mean ± SE plasma thromboxane (TBX [pg/mL]) in mallard ducks following i.m. injection of flunixin (FLX [5mg/kg], n = 5), ketoprofen (KET [5mg/kg], n = 6), and saline injected controls. *Significantly different from baseline values, P ≤0.05.

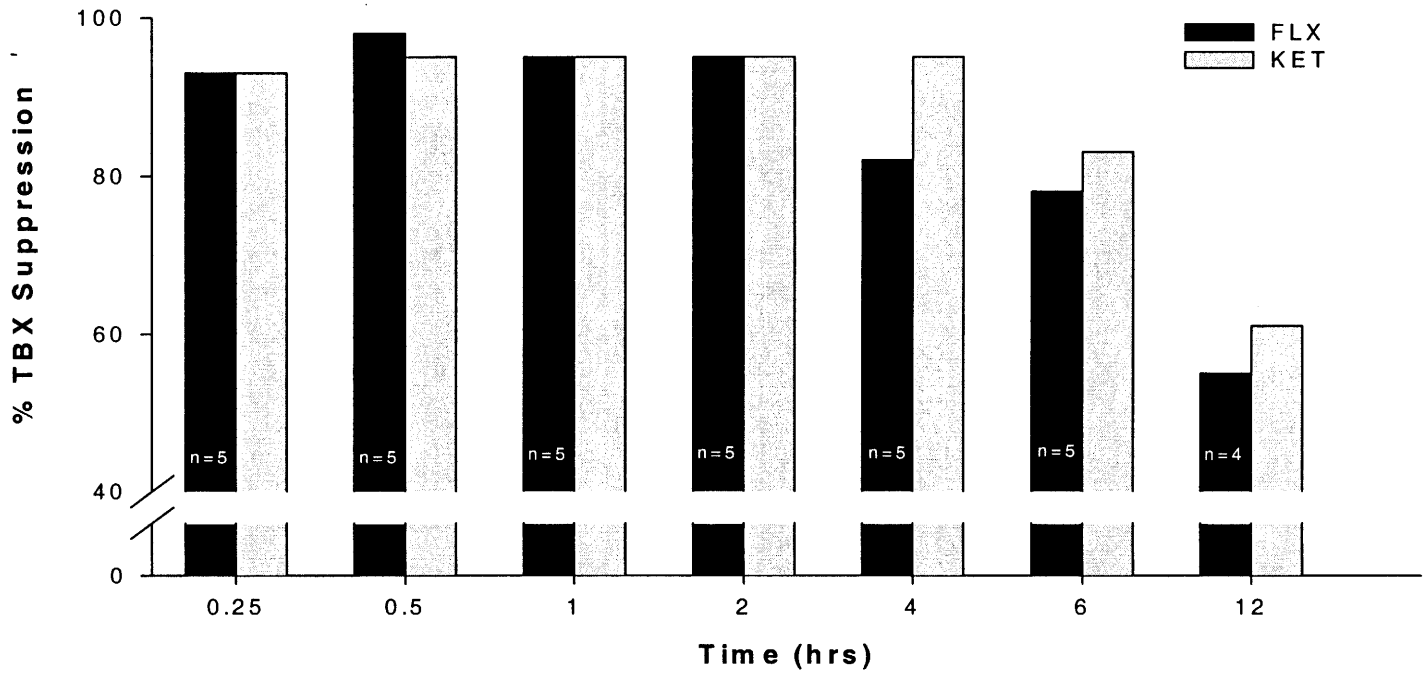

Figure 3.2. Mean percent thromboxane (TBX) suppression compared to baseline in mallard ducks following i.m. injection of flunixin (FLX [5mg/kg]), ketoprofen (KET [5mg/kg]). n = number of ducks suppressed

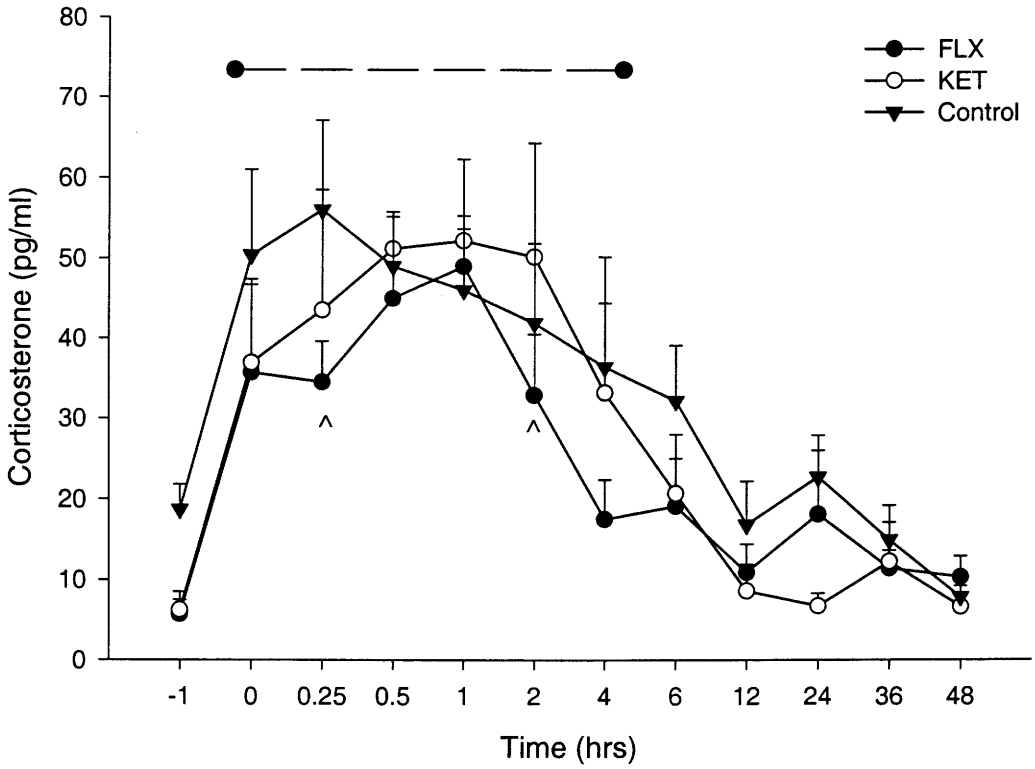

Figure 3.3. Mean ± SE plasma corticosterone (pg/mL) in mallard ducks prior to (-1 and 0 h) and following i.m. injection of flunixin (FLX [5mg/kg], n = 5), ketoprofen (KET [5mg/kg], n = 6), and saline controls (n = 4). All samples under the line (●——●) were significantly different from the first sample (-1 h) $P \leq 0.05$, except FLX at 0.25 and 2 h (^) where n = 2.

4. EXPERIMENTAL EVALUATION OF PLASMA BUPIVACAINE LEVELS IN MALLARD DUCKS FOLLOWING A SINGLE SUBCUTANEOUS DOSE

4.1 Abstract

Birds may be more sensitive to the toxic effects of local anesthetics than mammals but the pharmacokinetics of bupivacaine are unknown in birds. The purpose of this investigation was to determine the fate of bupivacaine following a single subcutaneous injection. Bupivacaine (2 mg/kg of a 0.5% solution) was given subcutaneously to eight adult female mallard ducks. Blood samples of 1.0 mL were drawn from the jugular vein prior to bupivacaine administration (0 h) and at 0.25, 0.5, 1, 2, 3, 4, 6, and 12 h after administration. Samples were assayed by high performance liquid chromatography and pharmacokinetic parameters were calculated from data plotted to the best-fit curve for samples from 15 to 180 min. The absorption time ($T_{1/2abs}$ = 13.9 min) appeared to be shorter than elimination time ($T_{1/2elim}$ = 28.1 min) which may, in part, explain why birds are susceptible to toxic effects of local anesthetics. Maximal plasma concentration (C_{max}) of 0.08 ± 0.06 (mean ± SD) μg/mL was observed (T_{max}) at 31.2 ± 0.06 min but high plasma levels were evident at 6 and 12 h (0.061 and 0.074

μg/mL, respectively). The appearance of subsequent high levels of plasma bupivacaine is unique to this study and may also contribute to the apparent avian sensitivity to toxic effects of local anesthetics.

4.2 Introduction

Local anesthetics such as lidocaine and bupivacaine function by blocking ion channels thereby preventing impulse conduction of pain (Courtney 1980). Local anesthesia before tissue trauma can significantly reduce postoperative pain as it prevents nociceptor sensitization and therefore avoids central changes that are secondary to activation of pain pathways (Coderre and Melzack 1987). In avian species, local anesthetics are often used without sedation or general anesthesia for minor surgical procedures, and for operative and/or post-operative pain relief in conjunction with general anesthesia for more involved procedures (Paul-Murphy and Ludders 2001). For example, bupivacaine was used to provide analgesia during anesthesia for laparotomies for placement of intra-abdominal radio transmitters in free-ranging ducks by infiltrating the surgical site on the ventral abdomen (Machin and Caulkett 2000).

In domestic chickens, bupivacaine has produced effective analgesia in two pain models. Chickens that had a bupivacaine/dimethyl sulphoxide mixture applied topically to the beak stump after amputation were able to maintain their pre-trimming feed intake levels for 4 h after the procedure compared to those that received no analgesic (Glatz et al. 1992), and intra-articular injection of bupivacaine in a sodium urate acute synovitis

model alleviated pain related behaviours (Hocking et al. 1997). Birds may be more sensitive to the toxic effects of local anesthetics than mammals, as lower doses (2.7 - 3.3 mg/kg) produce toxic effects in birds (Hocking et al. 1997) than in dogs (3.5 -4.5 mg/kg) (Skarda 1996). However, pharmacokinetics of local anesthetics in avian species are unknown. The objective of this investigation was to determine the fate of bupivacaine following a single subcutaneous dose.

4.3 Materials and Methods

Eight adult female mallard ducks, F2 progeny of wild strain adults, weighing 1060 ± 10 g, were used for the study. Ducks were given bupivacaine (2 mg/kg, 0.4 mL/kg of a 0.5% solution, Marcaine, Sanofi Winthrop, Chatham, Ontario, Canada) subcutaneously in the ventral abdomen to mimic location of local anesthetic administration required for surgical implantation of an intra-abdominal transmitter as previously described (Machin and Caulkett 2000). Administration of bupivacaine was between 0900 and 1100 h. Blood samples of 1.0 mL were drawn into heparinized syringes from the jugular vein before drug administration (0 min) and at 15, 30, 60,120, 180, 240, 360, and 720 min after administration. Samples were then placed in an integrated plasma separation tube with lithium heparin (Sherwood Davis and Geck Medical, St Louis, MO), then centrifuged and frozen at -20 °C until analyses were performed.

Samples were analysed following Gupta and Dauphin (Gupta and Dauphin 1994). Briefly, plasma samples were cleaned by solid-phase extraction on a column of Bond Elut, size 1 mL, with C18 sorbent material. Concentrations of bupivacaine in plasma were determined by high-performance liquid chromatography using a fluorescence detector and peaks were detected at 205 nm. Phenylcaine (N-pentyl-2,6 pipecolsylidine) was used as the internal standard. Quality control data at 5.0 μg/mL, mean (± SD) = 5.02 ± 0.15, CV = 3.01 %, at 0.312 μg/mL, mean (± SD) = 0.29 ± 0.02, CV = 7.9 %, and 0.05 μg/mL, mean (± SD) = 0.059 ± 0.003, CV = 5.12 %. The detection limit for the assay was 0.03 μg/mL.

Pharmacokinetic modelling was performed using the computer program WIN-NONLIN, version 1.1 (Statistical Consultant Inc. Lexington KY, USA). Standard pharmacokinetic parameters were calculated from data plotted to the best fit curve for samples from 15 to 180 min according to a one compartment first order, first order elimination model. Area under the curive (AUC 0 to 180 min) was computed by trapezoidal rule. All pharmacokinetic parameters were calculated for each animal and data are reported as mean ± standard deviation.

4.4 Results

One duck was excluded from the analysis as results were below detection limit of the assay from 15 to 180 min. The remaining data only fitted the model for up to 180 min. For this period the harmonic mean for absorption half-life ($T_{1/2abs}$) was 13.9 min

and for elimination half-life ($T_{1/2elim}$) was 28.1 min. Subcutaneous administration gave an $AUC_{(0\ to\ 180\ min)}$ of 0.14 ± 0.08 μg/h/L with a maximal plasma concentration (C_{max}) of 0.08 ± 0.06 μg/mL which was observed (T_{max}) at 31.6 ± 6.5 min. High levels were also evident in 5 of 8 ducks at 360 min (0.061 ± 0.05 μg/mL) and in 6 of 8 ducks at 720 min (0.074 ± 0.04 μg/mL) The plasma concentration vs. time distribution is shown in Figure 4.1.

4.5 Discussion

Metabolism of local anesthetics is very important because toxicity depends largely on the balance between rates of absorption and elimination (Catterall and Mackie 1995). In this study, the absorption rate was faster than the elimination rate which may, in part, explain why birds are more sensitive to toxic effects of local anesthetics compared with mammals. Chickens receiving high doses of intra-articular bupivacaine (2.7 - 3.3 mg/kg) had evidence of toxicity (recumbency, drowsiness) and signs of distress immediately after injection (Hocking et al. 1997). In dogs, plasma levels of 2.8 μg/mL are associated with increased QRS duration and conduction time (Freysz et al. 1989), but plasma levels of 1.5 - 2.3 μg/mL in humans can cause dizziness and drowsiness (Davies and Walford 1986). Plasma levels required for clinical signs of toxicity in birds are unknown, but in order to avoid any serious side effects 2 mg/kg was chosen for this study. Plasma levels attained in this study were well below levels required for toxicity in other species.

Systemic reactions to local anesthetics involve primarily the central nervous system (CNS) and cardiovascular system. In general the CNS is more susceptible to the actions of systemic local anesthetics and thus the doses required to produce CNS toxicity are usually lower than those resulting from circulatory collapse (Mather and Cousins 1979). Clinical signs can include depression, drowsiness, ataxia, nystagmus, and muscle tremors. Respiratory or metabolic acidosis can increase the risk of CNS toxicity in animals and humans (Englesson and Grevsten 1974).

The concentration of local anesthetics in blood is determined by the amount injected, the rate of absorption from the site of injection, the rate of tissue distribution, and the rate of biotransformation and excretion (Berde and Strichartz 2000). For most local anesthetics, there is a linear relationship between the amount of drug administered and the peak in blood concentrations (Covino and Vassalo 1976). Diffusion of local anesthetic solutions, injected subcutaneously, from a deposition site is a function of tissue binding and removal by circulation. The speed and extent of these processes depend on pK_a and on the lipophilicity of its base and cation species (Courtney 1980). The hydrophobicity gives an indication of drug delivery to the receptor while pK_a gives an indication of how well the drug will remain at the site (Ragsdale et al. 1994). Bupivacaine has a pK_a of 8.1 and hydrophobicity of 3420 which indicates that at physiologic pH, the percentage of cationic form would be approximately 80% (Covino and Vassalo 1976). Local anesthetics of greater hydrophobicity, like bupivacaine, are proportionately more potent (Courtney 1980).

It is difficult to estimate the activity of local anesthetics from blood concentration as the amount of drug present at the site determines local anesthetic efficacy. Bupivacaine is considered to have long duration of action in comparison to other local anesthetics. When used as infiltration anesthesia, it provides approximately 150 to 360 min of analgesia in dogs (Skarda 1996). Dose or concentration of the local anesthetic can alter onset and duration: for example, onset of action of 0.25 % solution of bupivacaine is slower and of shorter duration compared to 0.75% (Scott et al. 1980). The most rapid onset but the shortest duration of action occurs following intrathecal or subcutaneous administration of local anesthetics compared to intercostal, brachial plexus or epidural blocks. These differences are due in part to the particular anatomy of the area of injection, which influences the rate of diffusion and vascular absorption (Berde and Strichartz 2000). Bupivacaine alone does not greatly alter tissue blood flow when compared with saline alone (Dhuner and Lewis 1966). Although it is difficult to compare studies with different methodology, a 15 min i.v. infusion of 3.4 mg/kg bupivacaine in dogs had an elimination half-life of 39.1 ± 13.3 min (Arthur 1988). The length of action of bupivacaine in mallard ducks is possibly shorter as $T_{1/2elim}$ (28 min) is rapid; however, further studies are needed to confirm this.

In mammals, local anesthetics are distributed throughout all body tissue but relative concentrations in different tissues varies. Although skeletal muscle does not show any particular affinity for local anesthetics, the highest percentage of an injected dose is found in skeletal muscle because it is the largest reservoir (Arthur 1987). In mammals, local anesthetics are rapidly extracted by lung tissue resulting in marked

decreases in plasma concentration of local anesthetics. The extravascular pH of the lung is low relative to plasma pH, and produces ion-trapping of local anesthetics (Lofstrom 1978). This first-pass lung uptake consists of drug distribution not drug clearance. It is unknown if the avian lung is capable of similar extraction. The pH of arterial blood in mallard ducks is 7.46 (Machin and Caulkett 1998) indicating that birds are possibly more susceptible to ion trapping in the lung than mammals. However, uptake by the lung may be counteracted by greater plasma binding that occurs at higher pH values (Burney et al. 1978).

In this study, C_{max} of 0.08 ± 0.06 was observed at 31.6 ± 6.5 min but high levels were also evident at 360 and 720 min. The appearance of these high levels of plasma bupivacaine 360 and 720 min after administration have not been recorded in other species. Pharmacokinetics of bupivacaine can depend on time of administration (Bruguerolle and Prat 1987) and, as it is highly protein bound, it may be influenced by circadian variation in protein binding (Bruguerolle et al. 1983). In birds, the lung, and the pectoral and gastrocnemius muscles have relatively low perfusion rates at rest (Stephenson 1994) but during exercise, blood flow to these tissues can increase by 3 to 5 times (Butler 1988; Stephenson 1994). It is possible that periods of activity resulting in increased blood flow through the muscle and lung, with concurrent pH changes, may produce intermittent redistribution of bupivacaine. This may also contribute to avian sensitivity to local anesthetics and suggest that delayed toxicity may be possible.

Like lidocaine, bupivacaine is an aminoamide which undergoes hepatic metabolism by the cytochrome p-450 enzyme system. In mammals, excretion of

metabolites occurs via the kidney and less than 5 % of the unchanged drug is excreted via the kidney into the urine (Berde and Strichartz 2000). Like mammals, birds have an cytochrome p-450 enzyme system (Walker 1998) but comparisons of local anesthetic metabolism have not been done.

The amide-linked local anesthetics are extensively bound to plasma proteins, particularly α1-acid glycoprotein. Bupivacaine is 95.6% protein bound compared to 64.3% binding of lidocaine (Berde and Strichartz 2000). In mammals, many factors (disease, cancer, surgery, trauma, myocardial infarction, uremia) can increase the concentration of these plasma proteins and this appears to be similar in avian species (Inoue et al. 1997). In humans, increased plasma binding occurs with inflammatory disease in which plasma α1-acid glycoprotein concentration also rises as part of the stress response. Changes in α1-acid glycoprotein alters the amount of local anesthetic delivered to the liver for metabolism, thus influencing toxicity. In mammals, age related changes in protein binding can also occur because the neonate is relatively deficient in plasma proteins that bind local anesthetics and thereby have greater susceptibility to toxicity. In chickens, plasma α1-acid glycoproteins increase within 3 days after hatching and are similar to 7 week old chickens (Inoue et al. 1997; Takahashi et al. 1994). However, injection of *Escherichia coli* lipopolysaccharide decreases cytochrome P450 reductase activity in hepatic microsomes in male broilers (Takahashi et al. 1997). This suggests that local anesthetic binding to α1-acid glycoproteins may increase, while metabolism may decrease. As α1-acid glycoproteins concentrations were not determined in this study, it is unknown if changes in concentration affected the results of

this study.

This study suggests that bupivacaine may be shorter acting in ducks than it is in mammals. A shorter absorption time compared to elimination time may, in part, explain avian sensitivity to local anesthetics, however, more information on drug distribution is required to draw concrete conclusions. In addition, sequestration and redistribution of bupivacaine may result in a delayed toxicity but mechanisms are unknown. Pharmacokinetics may contribute to the sensitivity of avian species to local anesthetics but other possible mechanisms could be involved. The avian blood brain barrier is not as complex as that of mammals (Stewart and Wiley 1981) and this may allow for higher concentrations of local anesthetic in the brain. Further study is necessary to elucidate the mechanism of local anesthetic toxicity in avian species.

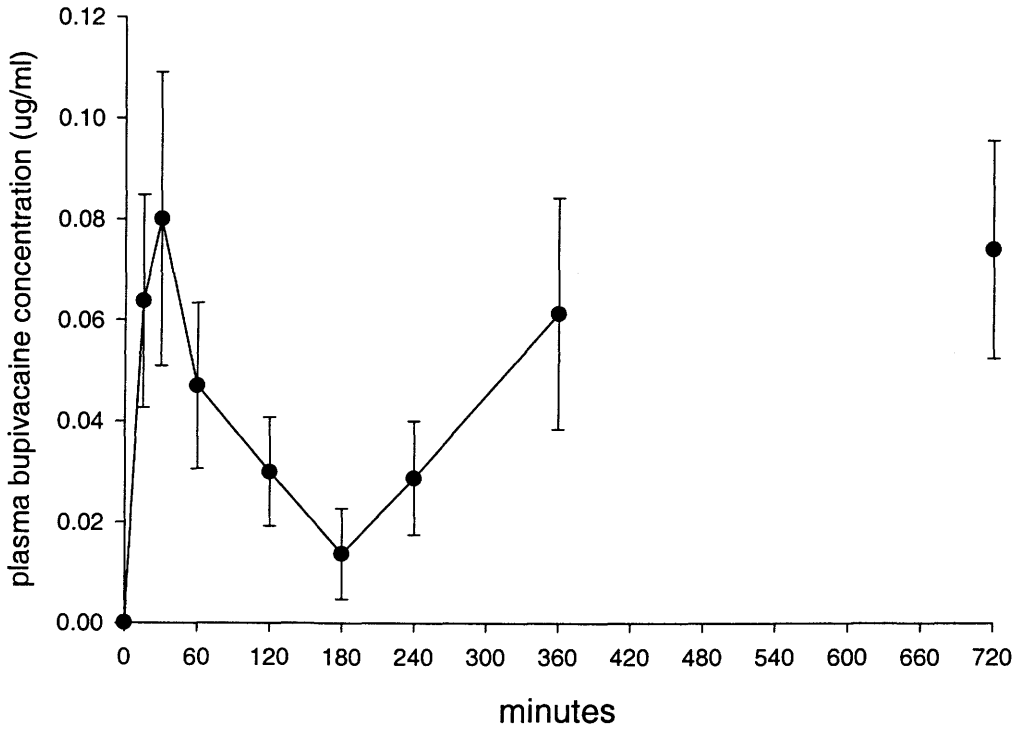

Figure 4.1. Mean (± SE) plasma concentration of bupivacaine (μg/mL) following subcutaneous injection of 2 mg/kg (0.4 mL/kg) 0.5% solution in 8 female mallard ducks. Samples at 360 and 720 min are increased in plasma bupivacaine concentration levels in 5of 8 and 6 of 8 ducks, respectively.

5. ASSESSMENT OF KETOPROFEN AS AN ANALGESIC IN ISOFLURANE-ANESTHETIZED MALLARD DUCKS

5.1 Abstract

Thirteen healthy, adult, wild-strain mallard ducks (*Anas platyrhynchos*) were used to determine whether administration of ketoprofen would have analgesic effects in spontaneously breathing ducks anesthetized with isoflurane. Each duck was anesthetized twice in a crossover study design with 6 days between treatments. Ducks were given ketoprofen (5 mg/kg, IM) or saline solution after a constant plane of anesthesia was established. Analgesia was assessed by measuring heart and respiratory rates and duration of application of a noxious stimulus. The noxious stimulus was applied 30, 50, and 70 minutes after drug administration and was maintained until gross purposeful movements were seen or for a maximum of 5 seconds. At all 3 evaluation times, heart rate increases in response to the noxious stimulus were greater when ducks were given saline solution than when they were given ketoprofen. The increase in respiratory rate in response to the noxious stimulus was greater when ducks were given saline than when they were given ketoprofen only 70 minutes after drug administration. When ducks were given ketoprofen, duration of the noxious stimulus was significantly longer 50 and 70 minutes, but not 30 minutes, after drug administration. Ketoprofen

reduced the increases in heart and respiratory rates associated with application of a noxious stimulus in spontaneously breathing adult Mallard ducks anesthetized with isoflurane delivered at approximately 2.9 %, suggesting that ketoprofen had analgesic effects in these ducks. The onset of analgesic effects may be longer than 30 minutes in some ducks.

5.2 Introduction

Prostaglandins (PGs) are important local mediators of inflammation and pain, and are also known to lower activation threshold to thermally, mechanically and chemically noxious stimulation. Nonsteroidal anti-inflammatory drugs (NSAIDs) control pain predominantly by inhibiting the cyclooxygenase enzyme which prevents the production of PGs. Drugs that inhibit PG biosynthesis in mammals produce analgesia by decreasing inflammation at the site of injury and also through central nervous system effects (Mathews 2000). PGs are involved in the modulation of avian pain responses and the physiological mechanisms involving PGs are similar to those described in mammalian models (Nicol et al. 1992).

Chickens given phenylbutazone applied topically to the beak were able to maintain their pre-trimming feed intake levels over the first 24 hours after the procedure (Glatz et al. 1992) and at levels higher than in untreated birds. Lame chickens preferentially select food containing carprofen compared to food without analgesics (Danbury et al. 1997); in another study, carprofen increased the speed and walking

ability of rapidly growing broiler chickens with chronic lameness, (McGeown et al. 1999). However, flunixin did not produce an isoflurane-sparing effect in parrots (4mg/kg i.m.) (Curro 1994) and ketoprofen (0.5 to 2.0 mg/kg i.m.) did not appear to provide analgesia after skin incision in mallard ducks (Machin 1998).

One method of evaluating analgesic effects of a drug is to measure responses to a noxious stimulus. However, stress responses may influence responses to noxious stimuli, and separation from social companions can alter pain perception in gregarious species (Frohm and Wallnau 1983, Jones and Harvey 1997). For examples, birds held and tested in familiar pens are significantly less able to cope with noxious stimuli than are birds tested in novel pens (Gentle and Corr 1995, Gentle et al. 1989, Sufka and Weed 1994). In addition, repeated exposure to noxious stimuli can induce tonic immobility in birds, which can reduce responsiveness for several seconds to several hours (Gentle and Hunter, 1990, Gentle et al. 1989) Mallard ducks available for use in this study were a wild strain and had not been habituated to handling. Thus, ducks were anesthetized with isoflurane during the study to reduce stress associated with handling, separation from the group, and manipulation and to allow for an uncomplicated assessment of responses to a noxious stimulus.

Changes in heart rate, mean arterial pressure, respiratory rate and tidal volume have been used as indications of acute pain (Gentle and Hunter 1990). Minimum alveolar concentration (MAC) has been used to measure the potency of inhalant anesthetics and refers to the concentration required to prevent gross purposeful movements to a noxious stimulus in 50 % of the animals. Analgesic potency of drugs have been assessed though the ability of an agent to reduce MAC (Quasha et al. 1980).

In those studies, hemostats were usually used to provide the painful stimulus by clamping the skin (Quasha et al. 1980) or, in the case of birds, a digit was clamped to at least the first ratchet (Concannon et al. 1995) which would probably produce tissue damage. Likewise, direct measurement of blood pressure in birds necessitates a cutdown incision to place arterial catheters (Concannon et al. 1995). Trauma and inflammation ensuing from this sort of tissue damage may result in nociceptive information reaching the spinal cord and resulting in central sensitization, which may induce prolonged changes in CNS function that might later influence responses to subsequent afferent inputs (Katz et al. 1992).

In this study, traumatic damage was limited by covering the ends of the hemostats with plastic tubing and bending the ends into a curved position to prevent complete closure. In addition, the stimulus was only applied up to a maximum of 5 seconds. As isoflurane anesthesia does not provide analgesia (Dohoo 1990), ketoprofen analgesia was assessed in isoflurane-anesthetized ducks compared to a saline injected control through non-invasive methods by measuring heart rate, respiratory rate, and duration of noxious stimulus required to induce gross purposeful movements.

5.3 Methods and Materials

Ducks were maintained and treated in accordance with guidelines of the Canadian Council on Animal Care as defined by the Guide to the Care and Use of Experimental Animals. This project was approved by the University of Saskatchewan

Animal Care Committee. The study was conducted using 13 healthy adult female mallard ducks (*Anas platyrhynchos*), F2 progeny of wild adults, weighing 1346 ± 183 g (mean ± SD).

Each duck was anesthetized twice, in a crossover study design, with a minimum of 6 days between randomized treatments. Anesthesia was induced using 5 % isoflurane in oxygen delivered by mask. After induction, the duck was intubated with a 3.0 mm non-cuffed endotracheal tube, and anesthesia was maintained using 2 to 3 % isoflurane in oxygen, through a non-rebreathing coaxial system. Oxygen flow was set at 1 L/min. All ducks were maintained in sternal recumbency on a warm water heating pad, and were covered with towels to maintain body temperature between (38 - 39 °C). Electrocardiogram (ECG) leads were platinum electrodes placed subcutaneously immediately after intubation in the left and right patagium and left inguinal region using platinum electrodes. An opening in an elbow adapter, positioned between the endotracheal tube and the anesthesia circuit, allowed passage of a thermocouple to monitor ventilation.

End-tidal isoflurane concentration was monitored with the use of a Poet IQ anesthetic gas monitor (Criticare Systems Inc., Waukesha, WI 53186-4054). A polyethylene catheter was inserted through the opening in the elbow adapter, to the distal tip of the endotracheal tube prior to each test period to measure isoflurane levels. The anesthetic gas monitor was calibrated, using room air and 1.5 % isoflurane (Anesthetic agent calibration gas, 1.5 % isoflurane Criticare Systems Inc., Waukesha, WI 53186-4054) prior to the start of each experiment. The aspiration rate through the anesthetic

monitor was set at 50 mL/min.

Isoflurane concentration was altered to maintain a constant plane of anesthesia. Depth of anesthesia was assessed by monitoring respiration, nictitating membrane response to a stimulus (moving of the eyelid), and response to non-painful stimulation (extension of the wings and legs). Spontaneous respiration was assessed as present, if ventilation occurred at regular intervals, respiration was considered periodic if there were periods of apnea (> 5 sec) between multiple breaths, and absent if the apnea was greater than 1 min. Nictitating membrane movements in response to the stimulus were graded as 0 = no movement, 1 = sluggish and membrane covered less than 50 % of the cornea, 2 = slow and membrane covered 100% of the cornea, 4 = fast, 5 = spontaneous movement (no stimulation). A response to wing and leg extension was assessed as present if there was retraction of the limb and/or if there was an obvious change in heart and/or respiratory rate. Isoflurane concentration was adjusted until a constant light plane of anesthesia could be maintained with the presence of spontaneous respiration, nictitating membrane movement in response to opening and closing of the eyelid equal to grade 2, and there was no response to a non-painful stimulus.

Ketoprofen (5 mg/kg) or an equal volume of saline was given i.m. into the pectoral muscles immediately after the duck was anesthetized and instrumented. A single investigator delivered the painful stimulus, recorded data, and was blind to treatments. Ducks were anesthetized at a constant light plane of anesthesia for 30 min, to allow for equilibrium between parabronchial gas, arterial blood and the brain (Quasha et al. 1980), before the first painful stimulus was applied. After the stimulus, a constant

light plane of anesthesia was re-established, then birds were kept anesthetized for 20 min for equilibration before subsequent stimuli were delivered. The stimulus was applied 3 times in total for each experiment.

The painful stimulus was accomplished with a set of hemostats. To limit traumatic damage the ends of the hemostats were covered with plastic tubing and were also bent into a curved position to prevent complete closure. The stimulus was applied to the right leg (mid point on the tarsal metatarsal bone) for the first experimental period and the left leg for the subsequent experimental period. Pressure was applied manually until a positive response occurred or for a maximum of 5 seconds. A positive response was determined when gross, purposeful movement ensued (i.e. flapping of wings, kicking the feet, or lifting of the head or neck) (Concannon et al. 1995).

Heart rate, respiration rate and length of the stimulus were recorded using a Grass polygraph, with paper speed was set at 5 mm per second. Data were analysed by an observer blind to the treatments. The heart rate (beats/min) was calculated over a period of approximately 5 sec by measuring the recording. Heart rate was determined for each animal at 0, 25 and 45 seconds prior to and following the stimulus at 30, 50 and 70 min after injection of either ketoprofen or saline. The mean of the 3 values obtained prior to the stimulus was used as the control heart rate for each duck at 30, 50 and 70 min after injection. Respiratory rate (breaths/min) was determined by counting the breaths for 60 seconds prior to and following the stimulus at 30, 50 and 70 min after injection. The duration of stimulus was determined by directly measuring from the recording. Response to the stimulus was graded as 0 = none (maximum 5 second stimulus applied),

1 = slow, 2 = immediate, and 3 = immediate and violent.

For each duck, the change in heart and respiratory rate was calculated by subtracting the mean control value from the heart rate obtained at 0, 25 and 45 seconds after the stimulus. Change in heart and respiratory rates, and length of the noxious stimulus were compared among treatments and time after injection using a general linear model analysis and repeated-measures analysis of variance (ANOVA). A sign test was used to determine if differences existed in the direction of the heart rate change among treatments. SAS (SAS Institute Inc., 1990) was used for data analyses and results were considered significant at $P \leq 0.05$.

5.4 Results

Fifty minutes after drug administration, 11 of the 13 ducks had higher heart rates immediately after application of the noxious stimulus (0 seconds) when given saline solution than when given ketoprofen; this proportion was significantly (sign test; $P = 0.011$) higher than 50%, the proportion expected on the basis of chance alone. However, at 30 and 70 minutes, the proportions of ducks that had higher heart rates immediately after application of the noxious stimulus when given saline solution than when given ketoprofen (9 of 13) were not significantly (sign test; $P= 0.113$) different from 50%. For all 3 applications of the noxious stimulus (30, 50, and 70 minutes), mean change in heart rate immediately after application of the noxious stimulus (0 seconds) was significantly ($P = 0.01$) greater when ducks were given saline solution than when they were given

ketoprofen (Fig 1). At 50 and 70 minutes, but not at 30 minutes, change in heart rate 25 and 45 seconds after application of the noxious stimulus was significantly ($P = 0.002$ and 0.013, respectively) different when ducks were given saline solution than when they were given ketoprofen. However, at all 3 times, a significant effect of time on change in heart rate was detected.

End-tidal isoflurane concentration was not significantly different between treatments. End-tidal isoflurane concentrations 30, 50, and 70 minutes after administration of ketoprofen were 2.9 ± 0.6%, 2.9 ± 0.5%, and 2.9 ± 0.5, respectively. Mean end-tidal isoflurane concentrations 30, 50, and 70 minutes after administration of saline solution were 2.9 ± 0.7%, 2.9 ± 0.7%, and 2.8 ± 0.7%, respectively.

Three ducks had periodic respiration after administration of saline solution or ketoprofen; therefore, data on respiratory rate for these ducks were excluded from analyses. For the remaining 10, change in respiratory rate was significantly ($P = 0.049$) greater when ducks were given saline solution than when ducks were given ketoprofen at 70 minutes, but not at 30 or 50 minutes (Fig 2).

At 50 and 70 minutes, but not at 30 minutes, duration of the noxious stimulus was significantly ($P = 0.03$ and 0.02, respectively) longer when ducks were given ketoprofen than when ducks were given saline solution (Fig 3). When response to the noxious stimulus was graded, the proportion of ducks that had a higher grade when saline solution was given than when ketoprofen was given was significantly different from 50% at 50 and 70 minutes ($P = 0.008$ and 0.035, respectively), but not at 30 minutes ($P = 0.113$).

Redness and indentation of the skin overlying the midpoint of the metatarsal bone were noticeable after the first application of the noxious stimulus. Skin was damaged after the third application of the noxious stimulus, and scarring was evident 6 days later.

5.5 Discussion

In this study, administration of ketoprofen reduced the increases in heart and respiratory rates associated with application of a noxious stimulus in spontaneously breathing adult female mallard ducks anesthetized with isoflurane delivered at approximately 2.9%. In addition, the mean time the noxious stimulus could be applied before gross purposeful movements were seen (up to a maximum of 5 seconds) was significantly longer when ducks were given ketoprofen than when they were given saline solution. I conclude, therefore, that administration of ketoprofen at a dose of 5 mg/kg to mallard ducks results in clinically detectable analgesia. However, use of clinical signs to determine plane of anesthesia in this study and hypercarbia associated with administration of high concentrations of isoflurane may have confounded the results. Thus, additional research may be needed to verify the efficacy of ketoprofen in birds.

Ketoprofen's effect on heart rate were less evident 30 minutes after administration, compared with effects detected 50 and 70 minutes after drug administration. Potentially, this may have been attributable to the low number of ducks in the study, which reduced the power to detect differences. Alternatively, the onset of

analgesia may have been longer than 30 minutes in some ducks because of individual variations in uptake and distribution of the drug. In a previous study (Machin 1998), administration of ketoprofen (5 mg/kg) resulted in > 82% suppression in thromboxane B_2 activity by 15 minutes, indicating that absorption was rapid. However, degree of analgesia does not necessarily correlate with degree of suppression of thromboxane B_2 activity, as NSAID exert their analgesic effects through a variety of peripheral and central mechanisms (Cashman 1996). Analgesia associated with mechanisms of action of NSAID not related to local prostaglandin synthesis may take longer to become fully active. Proposed mechanisms of action of NSAID unrelated to prostaglandin synthesis include alterations in spinal nociceptive processing through cellular or intracellular mechanisms possibly involving interference with G-protein-mediated signal transduction (Cherng et al. 1996), central activation of endogenous opioid peptides (Groppetti et al. 1988, Martini et al. 1984), blockade of the release of serotonin (Groppetti et al. 1988), and inhibition of excitatory amino acids involved in N-methyl-D-aspartate receptor activation (Smullin er al. 1990).

The proportion of ducks with higher heart rates immediately after application of the noxious stimulus (0 seconds) when given saline solution was lower than when given ketoprofen 70 minutes after drug administration than it had been 50 minutes after drug administration. The power to detect significant differences may have been low because of the small sample size. However, isoflurane induces dose-dependent respiratory depression (Ludders et al. 1998), and as ventilation was not controlled in this study, it is possible that $PaCO_2$ increased over time (McDonell 1996). In this study, end-tidal

isoflurane concentration was approximately 2.9%, and in a another study (Ludders et al. 1998), spontaneously breathing Sandhill cranes (*Grus canadensis*) anesthetized with isoflurane at 2 times the MAC in this species (approx 2.8% isoflurane) had $PaCO_2$ values > 100 mm Hg. Hypercarbia has been associated with increases in vagal tone and bradycardia (Bing et al. 1969) and may induce narcosis (Fisele et al. 1967), which may have lessened differences in the change in heart rate in response to the noxious stimulus. However, end-tidal isoflurane concentration did not differ when ketoprofen was given versus when saline solution was given. Therefore, the effects of hypercarbia on heart and respiratory rates and on responses to the noxious stimulus would have been the same for each treatment. Accordingly, we believe that significant differences between treatments represent a true analgesic effect of ketoprofen. However, these results should be confirmed with more controlled and defined conditions.

In this study, a significant difference between treatments in regard to change in respiratory rate was detected only 70 minutes after drug administration, and not at 30 or 50 minutes. This suggests that, under the conditions of this study, respiratory rate may not have been as good an indicator of analgesia as heart rate. In particular, hypercarbia may have blunted changes in respiratory frequency and volume in these ducks. In addition, although changes in tidal volume and in inspiratory and expiratory duration may indicate a response to pain in awake perching birds (Paul-Murphy et al. 1999), ventilatory volume and $PaCO_2$ were not measured. Minute volume may have provided more information than respiratory rate alone, provided $PaCO_2$ had been similar among ducks.

Although we attempted to reduce traumatic damage associated with the noxious stimulus by altering the hemostats and minimizing the duration of application, some tissue damage was evident. It is possible that tissue injury and inflammation could have made the ducks more sensitive to the second and third applications of the noxious stimulus (ie, at 50 and 70 minutes), as tissue injury and inflammation can effect the peripheral and central nervous systems in mammals and alter sensitivity to subsequent stimuli. This sensitization may be characterized by a lower threshold of activation, an increased response to noxious stimuli, a shorter response latency, a longer duration of response to stimulation, an increased response to a given stimulus intensity or spontaneous activity, and a spread of pain and hyperalgesia to uninjured tissue (Campbell et al. 1983). However, duration of application of the stimulus was significantly longer when ducks were given ketoprofen than when they were given saline solution, yet fewer changes in heart and respiratory rates were seen when ducks were given ketoprofen. This suggests that ketoprofen produced analgesia even if there was increased sensitivity 50 and 70 minutes after drug application.

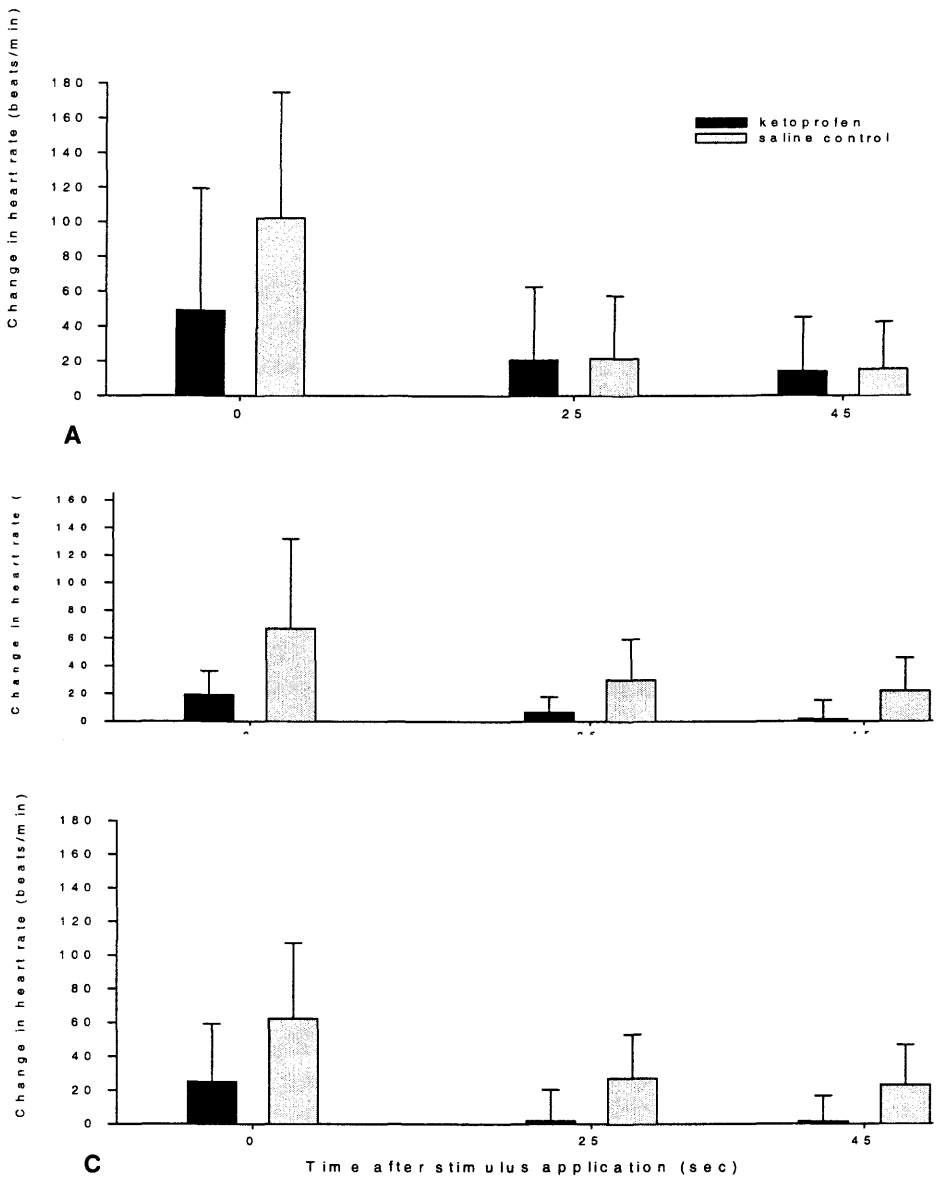

Figure 5.1. Change in heart rate (mean ± SD, beats/min) immediately (0 seconds) and 25 and 45 seconds after application of a noxious stimulus in 13 healthy, spontaneously breathing, adult female Mallard ducks anesthetized with isoflurane and given ketoprofen (5 mg/kg, IM) or saline solution. Responses were recorded 30 (A), 50 (B), and 70 (C) minutes after drug administration.

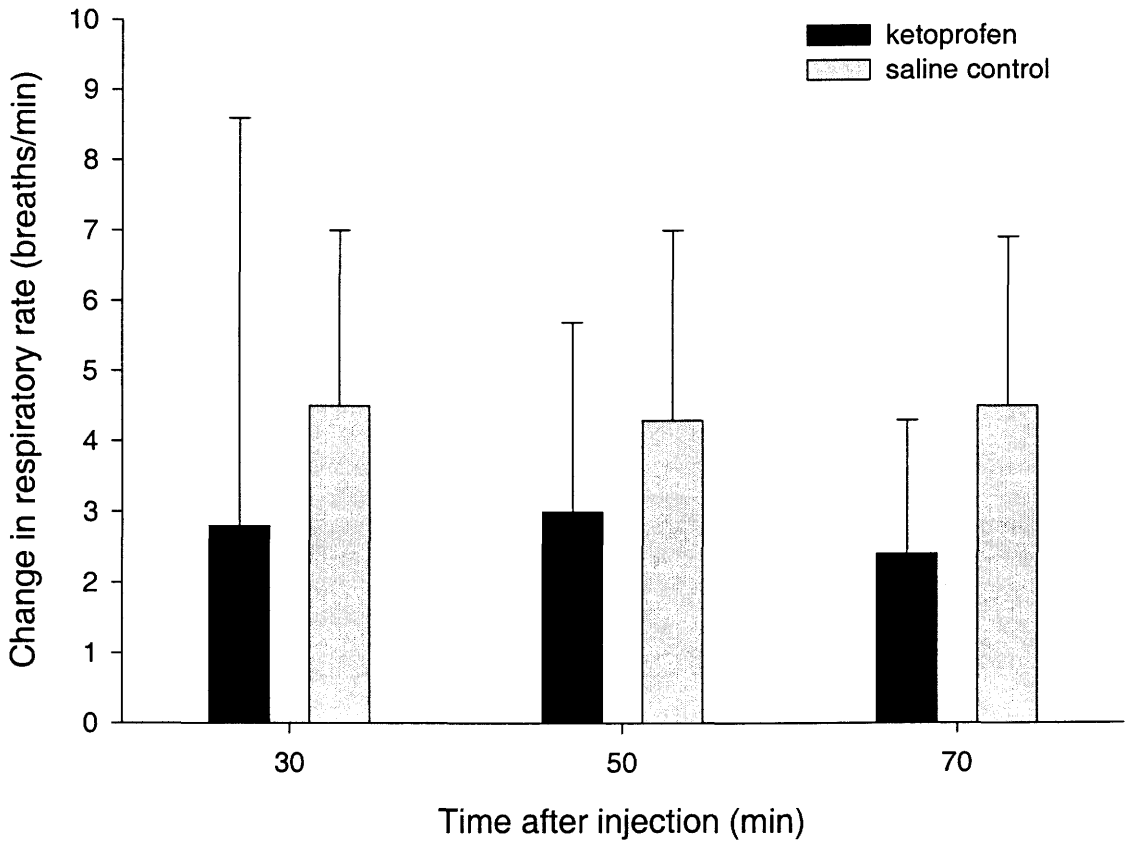

Figure 5.2. Change in respiratory rate (mean ± SD, breaths/min) in response to application of a noxious stimulus in 10 healthy, spontaneously breathing, adult female Mallard ducks anesthetized with isoflurane and given ketoprofen (5 mg/kg, IM) or saline solution; responses were recorded 30, 50, or 70 minutes after drug application.

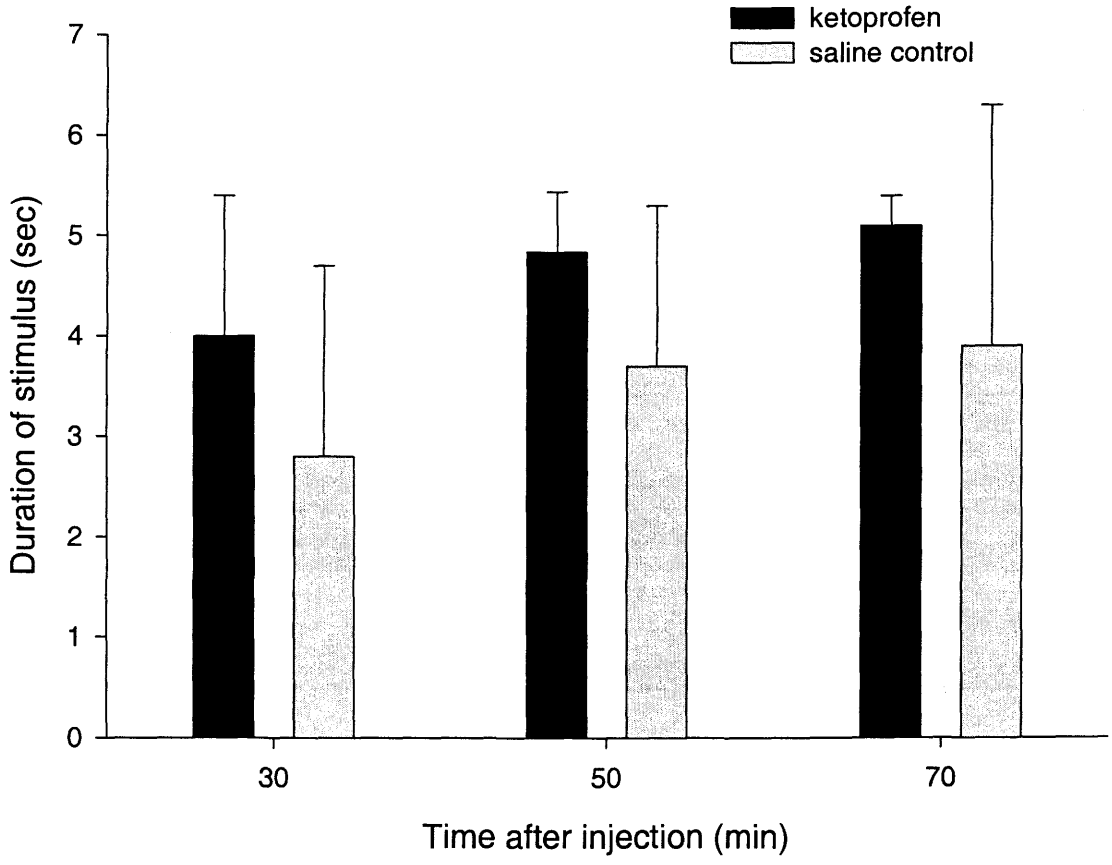

Figure 5.3. Duration of application of a noxious stimulus (mean ± SD) in 13 healthy, spontaneously breathing, adult female mallard ducks anesthetized with isoflurane and given ketoprofen (5 mg/kg, IM) or saline solution. The noxious stimulus was applied 30, 50, and 70 minutes after drug administration and was maintained until gross purposeful movements were seen or for a maximum of 5 seconds..

6. PHYSIOLOGICAL EFFECTS OF ANESTHETICS AND THE USE OF POST-OPERATIVE BEHAVIOUR IN ASSESSING ANALGESIA IN RUDDY DUCKS WITH INTRA-ABDOMINAL RADIO TRANSMITTERS

6.1 Abstract

Heart rate, occurrence of apnea, body temperature, and quality of anesthesia and recovery were compared in twenty male and seven pre-nesting female ruddy ducks (*Oxyura jamaicensis*) anesthetized with either propofol or isoflurane. The effect of surgery on free-ranging ruddy ducks was assessed by comparing post-operative behaviour of male ruddy ducks implanted with radio transmitters and given bupivacaine for analgesia with twenty males that were captured and leg banded. Heart rate, body temperature, quality of anesthesia and recovery was also assessed in seventeen incubating females anesthetized with propofol and given bupivacaine for analgesia in late incubation (17 to 23 days). Nest abandonment in incubating females was compared to a control group of sixteen females that were captured and leg banded. At the nest, females in the surgery group were given additional boluses of propofol until they were lightly anesthetized, whereas control birds were released unanesthetized. All birds survived surgery and anesthesia. Male ruddy ducks, shortly after arrival on the breeding grounds, required a larger total dose of propofol during anesthesia but recovered faster

compared to incubating females. Body temperature declined in all groups during surgery. Heart rates declined significantly over time in the isoflurane group and in incubating females anesthetized with propofol. Six hours after capture, nineteen males and four females that had surgery were located easily because they were resting motionless on open water or floating vegetation or on land, and had a puffed up appearance. One implanted male in the propofol group was killed by a red-tailed hawk approximately 6 hours after surgery. Males in the leg band only group were more difficult to locate and only eight of twenty male ducks in this control group were observed. No difference in behaviour was found between males anesthetized with isoflurane versus propofol. Overall, aspects of male behaviour between surgery and leg-banded-only ducks differed. Implanted males spent less time feeding and courting but more time resting than leg-banded-only males. Nest abandonment by incubating females did not differ between groups. Seasonal variation in propofol pharmacokinetics may occur in ruddy ducks. Bupivacaine did not provide long-lasting analgesia and changes in behaviour may make ducks implanted with radio transmitters more susceptible to predation.

6.2 Introduction

Radiotelemetry is utilized frequently in waterfowl research to study habitat use, reproduction, behaviour and survival. Intra-abdominal transmitters are often used preferentially over externally-mounted transmitters (Korschgen et al. 1984) as they

appear to have less effect on behaviour (Greenwood and Sargeant 1973; Pietz et al. 1993), survival (Paquette et al. 1997) or reproductive effort (Paquette et al. 1997; Pietz et al. 1993; Rotella et al. 1993). Intra-abdominal transmitters appear to provide more reliable data, however, surgery is required for radio transmitter placement (Korschgen et al. 1984; Olsen et al. 1992).

For surgical procedures, isoflurane is often the anesthetic of choice for birds because rapid reversal results in a wide margin of safety and is well tolerated by all species (Ludders et al. 1990). However, recovery from isoflurane-anesthesia is too rapid to allow placement of ducks into their natural environment and this may cause higher human-induced nest abandonment in incubating ducks (Machin and Caulkett 2000). Propofol is an intravenous agent that has been used to anesthetize ducks for placement of intra-abdominal transmitters, permits placement of animals into their environment before recovery, and can reduce nest abandonment following surgery (Machin and Caulkett 2000). However, as neither propofol (Thrumon et al. 1994) nor isoflurane (Dohoo 1990) provide operative or post-operative analgesia, supplemental analgesia is required.

Analgesia can be defined as the relief of pain without loss of consciousness. Analgesics function by decreasing stimulation of the ascending spinal pathways or by activating the endogenous descending pain modulation pathways (Clyde 1994). Pain is produced by any procedure or injury that causes tissue damage, and birds and mammals are likely to experience pain in a similar manner. Birds possess the neurological components to respond appropriately to a painful stimulus (Jones et al. 1985; Willis et al. 1979), have endogenous antinociceptive mechanisms to modulate pain (Bayon et al.

1980; Csillag et al. 1989; Reiner et al. 1984), and treatment with pharmacological agents used in mammals modulates pain pathways and behavioural responses to painful stimuli (e.g. Curro et al. 1994; Danbury et al. 1997; Glatz et al. 1992; Paul-Murphy et al. 1999). Pain perception allows an animal to minimize its exposure to potentially harmful stimuli (Clark 1995).

Birds do not indicate pain in an obvious manner because species that are preyed upon may be less likely to display overt pain-associated behaviour that may attract attention of predators (Livingston 1994). In birds, pain can be recognized by changes in posture (guarding of the affected area), temperament, or normal behaviour (i.e. reduced feeding or activity) (Jenkins 1993). Pain may also alter normal responses of a bird to its environment, which may increase susceptibility to predation. Modification of normal behaviour may result in erroneous interpretation of data if unrecognized subtle changes occur in the physiology, behaviour or welfare of the bird (Houston and Greenwood 1993). Trauma from surgery, direct pressure of the transmitter on internal organs or stretching of the peritoneum could cause pain and alter behaviour, but this has not been investigated in a field setting.

Bupivacaine is a local anesthetic and functions by blocking ion channels in neurons, thereby preventing generation and conduction of pain impulses (Plumb 1995). Local anesthesia applied before tissue trauma can significantly reduce postoperative pain because it prevents nociceptor sensitization and therefore avoids central changes that are secondary to activation of pain pathways (Clyde and Paul-Murphy 1999). When used as infiltration anesthesia, bupivacaine provides approximately 2.5 to 6 h of analgesia in dogs (Skarda 1996).

The objectives were to 1) compare the effects of propofol and isoflurane anesthesia during surgical placement of transmitters by monitoring induction, physiological parameters and recovery in ruddy ducks (*Oxyura jamaicensis*), and 2) determine the effects surgery by comparing post-operative behaviour of ruddy ducks implanted with radio-transmitters with those that did not have surgery.

6.3 Methods

All ducks were treated in accordance with guidelines of the Canadian Council on Animal Care as defined by the Guide to the Care and Use of Experimental Animals. This project was approved by the University of Saskatchewan Animal Care Committee. This study was conducted from May to July 1996 on a 15.4 km^2 study area near Minnedosa, Manitoba (50°10' N, 99°47' W). This study was done in conjunction with another study examining the mating system and reproductive success of male and female ruddy ducks (Brua 1998). Ninety-one (forty male, fifty-one female) ruddy ducks were monitored.

6.3.1 Anesthesia and Surgery

Anesthesia and surgery were performed as described by (Olsen et al. 1992) and (Machin and Caulkett 2000). Briefly, isoflurane was delivered through a non-rebreathing system by an Isotec 3 vaporizer (Ohmeda, BOC Health Care, West

Yorkshire, England). Anesthesia was induced with isoflurane starting at 1% and stepped up to 5% with an oxygen flow of 2 L/min. Ducks were intubated with a non-cuffed endotracheal tube and anesthesia was maintained between 1.5 to 3.5% with oxygen flow rate of 1 L/min during surgery. Birds that became apneic were ventilated with a 0.5 L rebreathing bag attached to the circuit. Propofol was delivered through an IV catheter placed in the medial tarsal vein and anesthesia induction was accomplished by administering 10 mg/kg of propofol slowly over one min. Additional boluses (1 to 2 mg/kg) were given until the bird could be intubated with a non-cuffed endotracheal tube and as needed throughout the procedure (Table 1). Ducks were ventilated throughout the procedure using a pediatric self-inflating resuscitation apparatus (ABMU bag). Anesthetic depth (for both anesthetics) was adjusted to maintain the bird at a constant level of anesthesia.

Birds were placed in dorsal recumbency and prepared for surgery. During preparation and surgery, depth of anesthesia was assessed by monitoring (a) heart rate using an esophageal stethoscope, (b) nictitating membrane movements, (c) swallowing or coughing, (d) response to stimuli and (e) movement. Quantity of anesthesia, heart rate, and response to stimuli were recorded by observers every 5 min for all birds receiving transmitters. After the anesthetic was discontinued, heart and respiratory rate were monitored until normal breathing resumed. The endotracheal tube was removed when the bird began to lift its head to cough or swallow. Respiration was monitored for a few minutes following extubation to ensure that ventilation was maintained.

Before surgery, the incision site of all birds was infiltrated with 2 mg/kg, 0.5% solution of bupivacaine (0.4 mL/kg, Marcaine, Sanofi Winthrop, Markham, Ontario Canada) to control operative and post-operative pain. When ambient temperature was less than 16 C, birds were placed on hot water bottles to aid in maintaining body temperature. A temperature probe, placed at least 10 cm into the esophagus was used to monitor body temperature. Body temperature was assessed immediately after induction and at termination of anesthesia. Time to complete surgery (surgical time), time from start to end of anesthesia administration (anesthesia time), time from end of anesthesia to removal of the endotracheal tube after normal breathing resumed and attempts were made by the duck to remove the tube (extubation time), and time from removal from the trap to time of release (total time held) were recorded.

6.3.2 Ruddy Ducks Early in the Breeding Season

Forty male and thirteen female ruddy ducks were captured using decoy traps (Anderson et al. 1980). All ducks were banded with standard US Fish and Wildlife Service aluminum bands and also received a unique coloured leg band on the opposite leg. Birds were assigned randomly an anesthetic for surgery; ten males and five females had surgery during isoflurane anesthesia whereas ten males and two females had surgery during propofol anesthesia. Twenty male and six female were leg-banded only. Different anesthetics were used to determine if recovery time or distance moved after recovery differed between an inhalant (isoflurane) or an injectable (propofol) anesthetic.

Transmitters weighed 18 to 20 g (25 mm x 42 mm, LTC-3, Advanced Telemetry Systems Inc., Isanti, MN, USA). After recovery, birds were placed in a cardboard box and left undisturbed for 20 min before release. Ducks in the control group were not anesthetized but handled for a similar amount of time.

Behavioural observations were performed on both transmitter and control groups, through a spotting scope at 6 h after surgery. As bupivacaine provides approximately 2.5 to 6 h of analgesia in dogs after infiltration (Skarda 1996), 6 h after surgery was chosen to assess the effectiveness of analgesia. Comfort movements (stretching, flapping wings, preening), resting in cover or in the open, swimming, foraging, courtship, and out of sight were recorded every 30 sec for 20 min.

6.3.2 Incubating Ruddy Ducks

Incubating female ruddy ducks were assigned randomly between surgically implanted with a radio transmitter (n = 17) or control (nest-trapped only, n = 16) groups. In most brood survival studies, females are equipped with transmitters during mid-to-late incubation to reduce the loss of equipment because of predation and to reduce the probability of nest abandonment (Gloutney et al. 1993). Therefore, female ruddy ducks were captured on the nest (Weller 1957) between 17 and 23 days of incubation of an average 23 day natural incubation period as determined by egg floatation (Brua and Machin 2000). Ducks were then implanted with radio transmitters during propofol anesthesia with bupivacaine infiltration of the surgical site. After recovery, birds were

placed in a cardboard box and carried to the nest site. At the nest, ducks in the propofol group were given additional anesthetic until depth of anesthesia was sufficient to allow removal of the catheter. Respiratory rate was monitored, bleeding was stopped by applying Blood Stop Powder (Dominion Veterinary Laboratories LTD., Winnipeg, Manitoba, Canada) and manual pressure. Ducks were allowed to recover on the nest without human disturbance. Post-operatively, females were located 1 or 2 times daily using a truck-mounted or hand-held receiving system. In the control group, sixteen female ruddy ducks were nest-trapped between 16 and 23 days of incubation. These birds were removed from the nest-site for measurements and marking but were released at the nest. Nest abandonment in both groups was determined by female location and nest visits.

6.3.4 Data Analysis

Paired t-tests were used to compare start and end body temperatures. Repeated measures analysis of variance (ANOVA) was used to evaluate variation in heart rate. Total dose of propofol, surgery time, anesthesia time, and time to extubation was compared between groups with a one-way ANOVA. A Fisher's exact test was used to examine nest abandonment rates among females that were anesthetized and received transmitters compared to females that were captured only. Aspects of male behaviour were analysed using a multivariate analysis of variance (MANOVA) to control simultaneously for several dependent behaviour variables. Due to small sample size,

female behavioural data were not analysed. If the MANOVA was significant, a univariate ANOVA was used to identify which aspects of behaviour varied between treatments. A MANOVA was used to determine if differences existed in behaviour between birds treated with the two anesthetics. SAS (SAS Institute Inc., 1990) was used for data analyses and results were considered significant at $P \leq 0.05$.

6.4 Results

All ducks survived anesthesia and surgery. Both propofol and isoflurane provided a smooth, rapid induction. Although there was no difference among groups in length of surgery or anesthesia, time to extubation was longer for incubating females than in any group caught during pre-laying ($F = 6.538$, $df = 4,38$, $p < 0.001$, Table 6.1). The total amount of propofol required to anesthetize incubating females was more than males ($F = 7.236$, $df = 2,25$, $P = 0.003$), but did not differ between pre-laying females and males. However, incubating females required relatively large doses of propofol for induction but very little for maintenance of anesthesia, whereas males required less propofol for induction but much higher doses to maintain anesthesia. Also, delivery rate of propofol in prelaying females appeared to be more similar to males than incubating females (Table 6.2).

Body temperature declined in all groups during surgery (Table 6.1) even though birds were placed on heating pads in cooler weather. Heart rate declined over time in males anesthetized with isoflurane (repeated measures ANOVA, $F = 3.89$, $df = 3,26$, $P =$

0.02). Although not significant, female heart rate in the isoflurane group also declined, but power to detect differences was low (repeated measures ANOVA, $F = 2.24$, $df = 3,12$, $P = 0.14$). In the propofol group, heart rate declined in incubating females (repeated measures ANOVA, $F = 17.48$, $df = 3,48$, $P < 0.001$) but was preserved in males (repeated measures ANOVA, $F = 0.45$, $df = 3,27$, $P = 0.72$ (Table 6.3). As with propofol, isoflurane-anesthetized birds required assisted ventilation since most birds developed apnea.

All ducks in the isoflurane group became apneic and required artificial ventilation and ducks in the propofol group were ventilated artificially regardless of spontaneous breathing. Ducks anesthetized with propofol recovered smoothly, while isoflurane-anesthetized ducks tended to struggle and flap wings upon recovery. One male ruddy duck anesthetized with propofol developed cardiac arrhythmia after premature extubation and apnea. The bird was re-intubated and ventilated until arrhythmia resolved. The bird was also given doxapram (Dopram-V, Ayerst Laboratories, Montréal, Canada) (5 mg i.v.) which stimulated respiration. The endotracheal tube was removed when the bird lifted its head and no further complications occurred. Also, recovery was prolonged (27 min) after propofol anesthesia in one incubating female ruddy duck that had soaked feathers from heavy dew and a broken egg. This bird was hypothermic (38.3 °C) at start of surgery and body temperature dropped to 37.2 °C despite being placed on a heating pad during surgery and recovery. Feathers were slightly damp when placed back on the nest, but the bird had no complications post-operatively. After 20 min in the cardboard box, birds in both groups

appeared alert and were able to swim away. One bird in the isoflurane group flew immediately off the pond. A female ruddy duck, recaptured 5 days after surgery, had a weight loss of 50 g but incision site was dry and had no evidence of inflammation.

6.4.1 Post-release Behaviour of Males

Six h after capture, nineteen males and four females that had surgery were located easily on the pond because they rested motionless on open water or on floating vegetation or land, and had a puffed up appearance. Identification was confirmed with the use of radio telemetry. Males in the leg-band only group were more difficult to locate and only eight of twenty ducks in this control group were observed 6 h after capture. One implanted male in the propofol group was killed approximately 6 h after surgery by a red-tailed hawk (*Buteo jamacensis*). The hawk was observed removing the carcass from the kill site.

No difference in aspects of behaviour were found between males anesthetized with isoflurane versus propofol (MANOVA, Wilks' $F = 1.12$, $df = 7,11$, $P = 0.42$). Therefore, results from these groups were combined to compare aspects of male behaviour between surgery and leg-banded-only birds. Aspects of male behaviour differed between surgery and leg-banded-only (MANOVA, Wilks' $F = 2.88$, $df = 7,19$, $P = 0.03$). Implanted males spent less time feeding and courting, but more time resting than leg-banded-only males (Table 6.4). Locomotion, comfort, and out of sight did not differ between treatments. Although not significant, leg-banded-only males tended to

rest more often in cover than males that had surgery (Table 6.4). In addition, four of 19 implanted males rested on floating vegetation in the open whereas leg-banded-only males always rested on the water.

Six h after surgery, one isoflurane-anesthetized male and two propofol-anesthetized males were located on different ponds from those which they were captured on. Twenty-four h after surgery two isoflurane-anesthetized females, one isoflurane-anesthetized male and two propofol-anesthetized males had moved from their capture ponds. The one isoflurane-anesthetized male could not be located and was assumed to be out of range.

6.4.2 Female Nest Abandonment

One of 17 (5.9%) females in the propofol group abandoned the nest after surgery. This nest was partially depredated (from 6 to 1 egg) within 2 days prior to surgery. In the control group, one of 16 (6.3%) females abandoned after capture. Although control birds were held for 16.0 ± 1.3 min compared to 59.6 ± 8.1 min for the surgery group, no difference in nest abandonment was found between groups (Fisher's exact test, P = 1.0).

6.5 Discussion

Propofol or isoflurane with bupivacaine infiltration at the surgical site provided excellent anesthesia for surgical placement of intra-abdominal transmitters. Total

propofol requirement during anesthesia was greater for males and pre-nesting females compared to incubating females. Gender differences in propofol metabolism have been described in humans. Female patients exhibit a larger peripheral volume of distribution, a higher metabolic clearance but reduced peripheral clearance compared to male patients (Vuyk et al. 2001), resulting in faster female recovery when equal doses are administered (Apfelbaum et al. 1993). In this study, birds captured early in the breeding season required a larger total dose of propofol but recovered faster than incubating females, indicating that distribution (Duke 1995) and/or metabolism (Hay Kraus et al. 2000) differed. To our knowledge, seasonal alterations in propofol pharmacokinetics have not been noted in other studies. Regardless of the mechanism, propofol should be given as required. Individual and species variation in propofol requirement is a disadvantage but tends to disappear as the administrator gains experience (De Grood et al. 1985).

Surgery and anesthesia resulted in a significant decline in body temperature. Disruption of thermoregulation during general anesthesia and heat loss through the abdominal incision and respiratory tract may be largely responsible. Hypothermia may also result in decreased metabolic rate (Leslie et al. 1995), which may, in part, explain the prolonged recovery of the wet female ruddy duck.

Ducks in the isoflurane group and incubating females given propofol had a decline in heart rate over time. This may be caused by a number of factors including direct cardiac depressant qualities of anesthetics (Claeys et al. 1988; Ludders et al. 1990; Sebel and Lowdon 1989), artificially elevated heart rates prior to induction from excitement, or apnea on induction resulting in hypoxia and tachycardia (Taylor et al.

1986). Artificial ventilation was necessary for both propofol (Goodman et al. 1987; Machin and Caulkett 1998) and isoflurane (Dohoo 1990; Ludders et al. 1990), as both produce dose-dependent ventilatory depression in mammals and birds. Propofol may preserve heart rate (Machin and Caulkett 1998), as seen in ruddy duck males that received propofol, but hypoventilation, especially on induction (Duke 1995), can result in hypercarbia, possible hypoxia and tachycardia (Kumar and Srivastava 1965). Initial excitement or hypoventilation and resultant tachycardia may explain decline heart rates over time seen in the incubating females.

Based on the pharmacokinetics of these agents in other species, it is unlikely that either propofol or isoflurane had residual effects that would affect post-operative behaviour beyond the immediate recovery period. Recovery times in dogs anesthetized for 11 to 35 min with propofol or isoflurane were similar. Dogs walked unaided after propofol-anesthesia within 7 to 27 (mean 13) min and after isoflurane-anesthesia within 10 to 20 (mean 12.5) min of termination of anesthesia (Peshin and Hall 1996). Despite a long elimination half-life, recovery from propofol is rapid because of redistribution and fast metabolism. Biliary excretion occurs in the dog with enterohepatic recycling and further sulfate conjugation, however, this recycling does not result in any clinical effects (Duke 1995). In comparison, metabolism of isoflurane is only 0.2%. Poor water solubility of isoflurane prevents equilibration of tissues with alveolar anesthetic partial pressure during short procedures. Recovery from isoflurane anesthesia is primarily through exhalation but there is also some redistribution. Length of recovery is normally a function of anesthetic duration.

Results from this study suggest that the surgical procedure produced abnormal behaviour post-operatively. Ruddy ducks are adapted to an aquatic lifestyle and resting on land is considered atypical behaviour (Brua 1998). Changes in posture (puffed up appearance), reduced feeding and courting activity are all behaviours which can be associated with pain (Jenkins 1993). These behaviours and increased time spent resting in the open may make ducks more susceptible to predation post-operatively. Local anesthesia using bupivicaine at the dosage used in this study probably provided analgesia during the surgical procedure but is likely not adequate for long-term pain control after surgical placement of intra-abdominal transmitters. Bupivacaine, being highly hydrophobic, has a tendency to remain at the infiltration site (Covino and Vassalo 1976) but may not provide complete analgesia to underlying muscle or peritoneum. Also, bupivacaine may not have as long a therapeutic action in ducks as it does in mammals (Chapter 4). Secondary inflammatory effects from surgery are likely longer lasting than the local anesthetic block and may have contributed to altered behaviour in male ruddy ducks and weight loss seen in the female ruddy duck. Garrettson and Rohwer (1998) also found that captive blue-winged teal (*Anas discors*) recovering from intra-abdominal radio transmitter surgery had progressive weight loss for one week.

Timely administration of analgesics is important as ongoing pain perception can have a negative effect on homeostasis and healing (Benson et al. 2000). Although long-term analgesia and prevention of post-operative pain-related behaviours were not achieved with bupivacaine, it does not preclude the possibility that there was some short-term or pre-emptive benefit. When dealing with conditions known to be painful in

humans it is essential to treat for pain (Flecknell 1988). Increasing the bupivacaine dose to attain longer lasting pain relief would not be beneficial as birds may be more sensitive to the toxic effects of local anesthetics compared to mammals, as higher doses (3.5 -4.5 mg/kg) (Skarda 1996) produce toxic effects in dogs compared with birds (2.7 - 3.3 mg/kg) (Hocking et al. 1997). Thus, bupivacaine dose should not exceed 2 mg/kg. Dilution of bupivacaine by 50 % may increase volume, thus allowing a greater area to be anesthetized (Slade 1994). However, higher concentrations produces a more rapid onset and prolonged sensory anesthesia (Scott et al. 1980).

Other analgesics have not been used to provide post-operative pain relief following intra-abdominal transmitter placement. Opioids (e.g. morphine) or alpha$_2$-agonists (e.g. xylazine) could be used to provide analgesia but residual sedation (Plumb 1995) may preclude their use in free-ranging waterfowl. The sedative effects can be reversed with drugs antagonizing the effects but analgesia would also be discontinued (Martin 1984). Nonsteroidal anti-inflammatory drugs (NSAIDs) do not have sedative effects (Plumb 1995) and may provide longer lasting analgesia by decreasing inflammation associated with the surgery and by their direct central nervous system effects (Dirig and Yaksh 1997; Mathews 2000). Pre-emptive administration of NSAIDs have the potential to result in kidney damage because they produce renal ischemia which results in reduced glomerular filtration rates (Harris 1992). Although a single pre-emptive dose of 5 mg/kg ketoprofen in either male or female mallard ducks did not produce renal lesions (Machin et al. 2001, Chapter 3) but care must be taken to avoid hypotension during anesthesia. It is clear that further research is necessary to evaluate pain and analgesia in free-ranging ducks.

Table 6.1. Temperature, total time for surgical procedure, total anesthesia time, time to extubation following end of anesthesia and total holding time for ruddy ducks using either intravenous propofol or isoflurane delivered in oxygen for surgical placement of intra-abdominal transmitters. Surgeries were performed after bupivacaine infiltration of the surgical site.

Anesthesia	Sex	n[a]	Start Temp. (C)	End Temp. (C)	Surgery Time (min)	Total Anesthesia Time (min)	Time to Extubation (min)	Total Holding Time (min)
Isoflurane[b]	M	10	39.2*[c]± 0.7[d]	38.7* ± 1.0	9.6 ± 1.6	17.6 ± 2.0	2.3 ± 1.4	74.6 ± 3.5
Isoflurane[b]	F	5	39.1 ± 1.3	38.6 ± 1.0	11.4 ± 3.1	20.4 ± 6.5	3.2 ± 1.8	74.0 ± 13.8
Propofol[b]	M	10	39.2* ± 0.6	38.7* ± 0.9	9.8 ± 2.7	19.0 ± 5.2	3.3 ± 3.7	72.5 ± 6.3
Propofol[b]	F	2	38.7 ± 0.2	38.5 ± 0	12.0 ± 0	18.0 ± 2.8	4.0 ± 1.4	73.5 ± 2.12
Propofol[e]	F	16	40.0* ± 1.0	39.1* ± 1.0	9.8 ± 1.5	15.2 ± 2.1	6.6 ± 2.3	59.6 ± 8.1

[a]Sample size.

[b]Implanted during pre-laying.

[c]Paired t-test, *P ≤0. 05, comparing start and end temperatures.

[d]Mean ± standard deviation

[e]Implanted during incubation

Table 6.2. Quantity of propofol (mg/kg) administered intravenously and isoflurane vaporizer settings (%) during anesthesia of ruddy ducks for surgical placement of intra-abdominal transmitters. Total amount of propofol (mg) given in twenty min of anesthesia is also given. Surgeries were performed after bupivacaine infiltration of the surgical site.

Anesthetic	Sex	n[a]	Time (min)				
			0 - 5	5 - 10	10 - 15	15 - 20	Total Drug Given (mg/kg)
isoflurane	M	10	3.7 ± 0.4[b]	3.5 ± 0.4	3.5 ± 0.3	3.3 ± 0.7	-------
isoflurane	F	5	3.8 ± 0.4	3.5 ± 0.4	3.3 ± 0.3	3.9 ± 0.9	-------
propofol	M	10	19.6 ± 4.1	11.9 ± 4.1	11.2 ± 7.4	9.7 ± 10.1	35.8 ± 3.2
propofol	F	2	21.2 ± 0.6	21.5 ± 3.2	7.8 ± 3.0	10.7 ± 1.8	60.9 ± 8.7
propofol	F	16	33.1 ± 2.5	0.8 ± 1.8	1.2 ± 1.9	0.6 ± 0.9	52.7* ± 18.0

[a]Sample size

[b]Mean ± standard deviation.

*Significantly different from males ($P < 0.05$) (One-way ANOVA)

Table 6.3. Heart rates (beats/min) during anesthesia (intravenous propofol or isoflurane delivered in oxygen) of ruddy ducks for surgical placement of intra-abdominal transmitters. Surgeries were performed after bupivicaine infiltration of the surgical site.

Anesthetic	Sex	n[a]	Time after induction (min)			
			5	10	15	20
isoflurane	M	10	261 ± 31[b]	253 ± 43*	237 ± 41*	233 ± 41*
isoflurane	F	5	265 ± 30	250 ± 23	233 ± 27	221 ± 35
propofol	M	10	228 ± 25	227 ± 32	240 ± 40	233 ± 48
propofol	F	2	276 ± 34	258 ± 31	234 ± 50	261 ± 4
propofol	F	16	260 ± 25	243 ± 27*	228 ± 23*	224 ± 14*

[a]Sample size

[b]Mean ± standard deviation.

*Significant decline from first time period (5 min, $P \leq 0.05$) (repeated measures ANOVA)

Table 6.4. Behaviour, as a percentage of observations (mean ± SD), of male ruddy ducks following surgery (n = 19) to implant a radio-transmitter compared with leg-banded-only males (n = 8). Results from males that were anesthetized with either propofol or isoflurane were pooled as there was no difference in behaviour of ducks between anesthetics. All ducks that had surgery were infiltrated with 2 mg/kg bupivacaine at the surgical site. Resting in cover is given as a percentage of the total resting observations.

Behaviour	Surgery	Banded-only	F*	P
Feeding	4.5 ± 8.4	26.6 ± 22.6	11.86	0.005
Locomotion	12.3 ± 15.6	15.9 ± 12.7	0.93	0.34
Comfort	10.0 ± 15.1	5.6 ± 5.3	0.12	0.73
Resting	67.2 ± 29.1	36.8 ± 28.8	7.16	0.02
Courting	0.3 ± 1.4	5.9 ± 11.0	4.04	0.05
Out of Sight	7.2 ± 14.9	8.1 ± 20.1	0	0.98
Resting in Cover	33.3 ± 38.0	66.8 ± 42.4	2.63	0.1

*Post-hoc one way ANOVA comparisons after significant MANOVA.

7. ASSESSMENT OF KETOPROFEN FOR POST-OPERATIVE PAIN RELIEF FOLLOWING INTRA-ABDOMINAL TRANSMITTER PLACEMENT IN FREE-RANGING PRE-NESTING MALLARD DUCKS

7.1 Introduction

Radiotelemetry is utilized widely in waterfowl research to study habitat use, reproduction, behaviour and survival. Intra-abdominal transmitters are often used preferentially over externally-mounted transmitters (Korschgen et al. 1984) as they appear to have less effect on behaviour (Greenwood and Sargeant 1973; Pietz et al. 1993), survival (Paquette et al. 1997) or reproductive effort (Paquette et al. 1997; Pietz et al. 1993; Rotella et al. 1993). However, anesthesia and surgery is required for radio transmitter placement (Korschgen et al. 1984; Olsen et al. 1992). Isoflurane is often the anesthetic of choice for birds because rapid reversal results in a wide safety margin, and is well tolerated by all species (Ludders et al. 1990). Isoflurane is usually administered without supplemental analgesia for transmitter placement (Korschgen et al. 1984; Olsen et al. 1992) even though isoflurane does not provide operative or post-operative analgesia (Dohoo 1990).

Birds possess the neurological components to respond appropriately to a painful stimulus (Jones et al. 1985; Willis et al. 1979), have endogenous antinociceptive

mechanisms to modulate pain (Bayon et al. 1980; Csillag et al. 1989; Reiner et al. 1984), and treatment of birds with pharmacological agents used in mammals modulates pain pathways and behavioural responses to painful stimuli (e.g. Curro et al. 1994; Danbury et al. 1997; Glatz et al. 1992; Paul-Murphy et al. 1999). An animal's ability to perceive pain allows it to minimize exposure to potentially harmful stimuli (Clark 1995). However, modification of normal behaviour may result in erroneous interpretation of data if subtle changes occur in the physiology, behaviour or welfare of the bird (Houston and Greenwood 1993). As pain can be produced by any procedure or injury that causes tissue damage, waterfowl implanted with radio transmitters likely experience pain.

Analgesia can be defined as the relief of pain without loss of consciousness. Analgesics function by decreasing stimulation of the ascending spinal pathways or by activating the endogenous descending pain modulation pathways (Clyde 1994). Analgesics such as opioids (e.g. morphine) or alpha$_2$-agonists (e.g. xylazine) could be used to provide analgesia, but residual sedation (Plumb 1995) may preclude their use in free-ranging waterfowl where prolonged recovery may be impractical and may increase predation risk or risk of disrubpted pair bonds. Nonsteroidal anti-inflammatory drugs (NSAIDs) and local anesthetics do not have sedative effects. Bupivacaine has been used to provide analgesia following intra-abdominal transmitter placement but long-lasting analgesia (as judged by post-operative behaviour) was not achieved in free-ranging ruddy ducks (Chapter 6). Nonsteroidal anti-inflammatory drugs inhibiting the cyclooxygenase enzyme which prevents the production of prostaglandins (PGs). Drugs that inhibit prostaglandin biosynthesis in mammals produce analgesia by decreasing

inflammation at the site of injury and also through central nervous system effects (Mathews 2000). Prostaglandins are involved in the modulation of avian pain responses and the physiological mechanisms involving PGs are similar to that described in mammalian models (Nicol et al. 1992).

Ketoprofen is a nonsteroidal anti-inflammatory drug that is used to provide analgesia in many mammalian species (Mathews 2000). Nonsteroidal anti-inflammatory drugs demonstrated efficacy in some avian pain models (Danbury et al. 1997; Hocking et al. 1997), but the efficacy of ketoprofen has not been investigated in avian species, especially in free-ranging animals.

My objectives were to determine long-term effects and safety of pre-emptive ketoprofen by comparing its effects on post-operative nesting success and survival of female mallard ducks implanted with radio-transmitters with controls given saline.

7.2 Materials and Methods

All procedures were in accordance with guidelines of the Canadian Council on Animal Care as defined by the Guide to the Care and Use of Experimental Animals and were approved by the University of Saskatchewan Animal Care Committee. Work was conducted from April to August 1998 at four, 65 km^2 study areas in the prairie pothole region of central Canada. The study areas were located near Donalda, Alberta (52°55' N, 112°6' W) , Farrerdale, Saskatchewan (51°52' N, 105°87' W), Jumping Deer Creek, Saskatchewan (51°23' N, 104°13' W), and Minnedosa, Manitoba (50°18' N, 99°87' W).

Pre-laying female mallard ducks were decoy trapped (Sharp and Lokemoen 1987) during April 1998 and randomly assigned to receive either 5mg (0.05ml) ketoprofen (Anafen, Rhône Mérieux Inc. Athens, Georgia) intramuscularly (IM) or 0.05 mL saline IM. Forty females from three sites and thirty-nine females near Donalda, Alberta were anesthetized with isoflurane and implanted with a 22 g transmitter (Telonics, Inc., Mesa, AZ; Model IMP/150), as previously described by Olsen et al (Olsen et al. 1992). Time of ketoprofen delivery, surgeon and length of surgery were recorded. Ketoprofen or an equal volume of saline was injected shortly before surgery and observers were blind to treatments. Prior to release, all ducks were weighed to the nearest 10 g using Pesola spring scales. Structural measurements of the wing (flattened wing chord), head, and tarsus length to the nearest 0.1 mm were taken. The second greater covert feather was removed from one wing and used to classify femalesas yearlings (second year) or older (after second year) (modified from (Krapu et al. 1979)).

After transmitter placement, females were located twice daily by triangulation when they were most likely to be on their nests (between 0600 and 1300, Gloutney et al. 1993). Triangulation was conducted using a vehicle-mounted null-array antenna system (4- and 5- element Yagi antennas: (Kenward 1987)). A female triangulated to the same location for 3 consecutive mornings was approached on foot with a hand-held receiving antenna to determine location of the nest. To reduce nest abandonment caused by investigator disturbance, nests were visited when the hen was absent from the nest. Each nesting female was located once daily, but if the female was absent from her nest, a second location was obtained later the same day. If the female was absent from her nest

for 2 consecutive telemetry locations, nests were visited to determine fate (active, abandoned, hatched or destroyed). If the nest was abandoned or destroyed, the female was again located twice daily to identify re-nesting attempts.

Data collected from radio tracking were used to estimate various measures of reproductive effort for each marked female: nest initiation date, number of days from surgery to nesting, number of nesting attempts, number of days devoted to egg laying and incubation, and nesting success, where ≥ 1 egg hatched. Hen survival, distance hen moved within a day, and distance moved from trapping site over the 3 days post-operatively were also determined.

Females whose nest was found destroyed and females that did not nest at all were excluded from comparisons related to nesting. Females that abandoned a nest due to investigator disturbance were eliminated from analysis of nest duration. Any female whose location was undetermined for ≥ 5 consecutive days during the were not considered monitored successfully and were excluded from analysis. Females with unknown hatch fate were not included in nest success analysis.

Results are reported as means $\pm$ SD. Principal components analysis was performed on three morphological variables (wing, head and tarsus length) to create an index of female body size. The first principal component (PC1) explained 48 to 68 % of the variation in body size with each variable loading positively. An index of female body condition was generated by using the residuals from the regression of female mass on PC1 for each female, thereby adjusting mass of each female for structural size. To remove study area as a confounding factor, initiation dates were standardized by

designating the first nest initiated at each site as day 1 and subsequent nests were designated to the number of days after the first nest was initiated. A general linear model (GLM) was used to determine if treatment, female age and body condition, and length of surgery (nested within surgeon) influenced reproductive data (days from surgery to initiation, nest initiation date, days spent in laying and incubation, and number of nesting attempts). Logistic regression (Proc CATMOD) was used to determine effects of treatment (ketoprofen or saline) and length of surgery (nested within surgeon), on the likelihood of nesting and female survival, controlling for female age and body condition. SAS (SAS 1990) was used for data analysis, results were considered significant when $P \leq 0.05$ and actual P values are reported.

7.3 Results

All sites has > 1 surgeon: Donalda, AB (2); Farrerdale, SK (2); Jumping Deer Creek, SK (5); and Minnedosa, MB (6). One female (1.3 %) in the ketoprofen group bled more from the muscle than was noted in other females but this female nested 20 days after surgery. Another female in the saline group had slight bleeding from the skin incision. One female in the saline group required very high levels of isoflurane (4.5 %) to maintain anesthesia and another was noted to have mild convulsions on recovery. After controlling for differences between age classes (partial F = 15.13, df = 1, 156, P = 0.0001), body condition did not differ between treatments (partial F = 0, df = 1, 156, P = 0.9603) or study area (partial F = 0.27, df = 3, 156, P = 0.8503).

Hen location (distance from the trap site) within the first 24 hours after surgery (950 ± 929 m) for females in the ketoprofen group did not differ from that (974 ± 602 m) in the saline group (Mann-Whitney Rank Sum test, T = 1510, n = 38 and 47, P = 0.28). Average daily distance moved over 3 days also did not differ between groups, where ketoprofen-treated females averaged 587 ± 302 m and the saline-treated females averaged 606 ± 234 m (Student's t test, t = -0.214, df = 34, P 0.831).

The proportion of females that nested did not differ between treatments (χ^2 = 0.78, df = 1, P = 0.38) nor did nest success (χ^2 = 0.67, df = 1, P = 0.43, Table 7.1). No difference between ketoprofen- or saline-treated groups was found in number of nests initiated (F = 0.62, df = 1,79, P = 0.43), days from surgery to initiation of the first nest (F = 0.58, df = 1, 104, P = 0.45), initiation date (F = 0.20, df = 1,88 , P = 0.65), or days spent in laying and incubation (F = 0.36, df = 1, 78, P = 0.55, Table 7.2). The likelihood of initiating a nest did not differ between treatment (χ^2 = 0.78, df = 1, P = 0.38), but was positively related with length of surgery (χ^2 = 0.7.55, df = 1, P = 0.008). Similarly days from surgery to initiation of the first nest was longer (3.5 days) for saline-treated females than ketoprofen-treated females, although non-significant (F = 0.58, df = 1, P = 0.45). Length of surgery was positively correlated with number of days to initiation of the first nest (GLM, F = 1.70, df = 1,.90, P = 0.05).

Hen survival did not differ between treatments (χ^2 = 0.00, df = 1, P = 0.99) and survival was greater than 13 days in all cases. Similarly, no difference between treatments was found in number of days surviving following surgery (F = 0.76, df =1, 20, P = 0.39, Table 7.3).

7.4 Discussion

No differences in reproductive effort, hen survival or distances moved post-operatively were detected between pre-laying female mallards that received ketoprofen or saline prior to surgery. However, the addition of ketoprofen to the protocol for implanting radio-transmitters into pre-laying females did no harm and may have provided a slight but non-significant advantage over females that did not receive analgesia. Ketoprofen-treated females nested 3.5 days earlier after surgery and initiated more nests per female than saline-treated females, but had similar nesting success and female survival rates. While results were not significant, power to detect differences was low, and may have resulted in a type II error. Differences between treatments were not large and as all reproductive parameters were obtained several days after surgery, therefore, measures of reproductive effort likely do not truly reflect ketoprofen efficacy. Because the physiologic effects of ketoprofen last approximately 12 h (Machin et al. 2001, chapter 3), the response variable we measured, which were collected over days or weeks post-surgery, may be less sensitive for detecting effects of ketoprofen than more immediate measures. For example, analgesia probably has very little impact on days that a female devotes to egg laying and incubation or nest success, whereas the number of days it took for a female to nest following surgery may be more meaningful because it estimates recovery. Evaluation of analgesia, by assessing behaviour with direct observation within hours of surgery may provide more insight into effectiveness of ketoprofen in providing post-operative analgesia (Chapter 6).

In another study (Machin et al. 2001, chapter 3), administration of ketoprofen (5 mg/kg) achieved > 82 % thromboxane B_2 (TBX) suppression by 15 min, indicating that absorption and distribution of ketoprofen was rapid. However, the degree of TBX inhibition cannot be correlated absolutely with the degree of analgesia and significant analgesia may not be present in all animals until 30 min after injection (Chapter 5). As nonsteroidal anti-inflammatory drugs exert their analgesic effect through a variety of peripheral and central mechanisms (Cashman 1996), analgesia brought about by some mechanisms (not related to peripheral PGs) may take longer to become fully active. In this study, ketoprofen was administered immediately before anesthesia and < 15 min prior to surgery. Mammalian studies show that pain-induced neural changes can be prevented by administration of analgesic agents before development of injury induces spinal hyperexcitability and associated pain related behaviours (Coderre et al. 1990; Katz et al. 1992). Because of the close proximity of analgesic administration and surgery it is possible that some pain-induced neural changes where not prevented, resulting in small differences between ketoprofen and saline controls. However, administration of analgesics earlier is likely not practical in field studies.

Increased surgery time was correlated positively with likelihood of nesting and the number of days to nest initiation. Wound infection rates in elective surgical procedures in dogs and cats were higher in procedures performed by student surgeons compared to more experienced veterinarians (Vasseur et al. 1988), most likely because student surgeons have longer surgery duration and less-refined tissue handling techniques. Increased tissue trauma is associated with more inflammation and may

compromise the ability of tissue to resist infection (Vasseur et al. 1988). Longer surgery duration has been correlated with increased cortisol concentration in cats undergoing ovariohysterectomy and may require additional post-operative pain relief (Smith et al. 1999). As ketoprofen produces some of its analgesia through anti-inflammatory effects, it may allow females to recover and initiate a nest faster following surgery compared to saline controls. Females that have the ability to produce a clutch, rely on endogenous nutrients (Krapu 1981) and thus, post-operative behaviour may have little influence on determining whether a female will nest. Increased time correlated with the likelihood of initiating a first clutch is likely spurious.

Anesthesia without the provision of analgesia results in increased requirement for inhalant anesthetic to maintain a surgical plane of anesthesia (Pascoe 2000). One female in the saline group required very high levels of isoflurane (4.5%) to maintain anesthesia. Inhaled anesthetics can be hyperalgesic at very low concentrations (i.e., concentrations that would be obtained at some point during recovery from anesthesia) by enhancing C-fibre activity (Zhang et al. 2000). Patients may perceive noxious stimulation to be more intense than if no anesthetic were present (Zhang et al. 2000). Birds are known for a violent recovery from inhalant anesthesia (Ludders and Matthews 1996) and it is possible that some of this behaviour during recovery may be attributable to intense pain. The "mild convulsions" noted in one female on recovery could have been writhing in response to pain. Provision of appropriate analgesia may help improve recovery.

Nonsteroidal anti-inflammatory drugs can effect bleeding time by cyclooxygenase enzyme inhibition resulting in reduced synthesis of thromboxane B_2 (Petrusewicz et al. 1995). Ketoprofen results in reduction of thromboxane B_2 levels for

at least 12 hours in both male and female mallard ducks (Machin et al. In Press), Chapter 3). Pre-emptive administration of ketoprofen may have prolonged bleeding time of one female but incidence of bleeding did not differ from that of the saline treated females. Also, no further complications were noted in the female with the prolonged bleeding. However, as blood loss was not common during surgical placement of intra-abdominal transmitters and did not result in post-operative complications in this study, there is no disadvantage to administering pre-emptive ketoprofen.

Females that received ketoprofen took 3.5 days less to initiate nesting compared with females that received saline, indicating that analgesia was beneficial and there was no evidence to suggest that ketoprofen was harmful. However, ketoprofen may have more benefit if administered well in advance of surgery, to allow time for the drug to exert its analgesic effects. As isoflurane offers no operative or post-operative analgesia (Dohoo 1990) and birds have the capacity to experience pain (Gentle 1992), it is appropriate to provide analgesia when dealing with conditions known to be painful in humans (Flecknell 1988). Pre-emptive ketoprofen may provide appropriate analgesia but more studies are required to determine its efficacy in post-operative pain management in mallard ducks following intra-abdominal transmitter placement.

Table 7.1. Number of females that nested (%), number of nests per female (mean ± SD), and nest success of female mallards (%) at four sites in prairie Canada following surgery to implant an intra-abdominal radio transmitter during isoflurane-anesthesia with either ketoprofen or saline given intramuscularly.

Study Area	Treatment	n	Number nested	Nest per hen	n	Nest Success
Donalda, AB	ketoprofen	10	8 (80)	1.5 ± 1.1	11	1 (9)
	saline	5	4 (80)	1.0 ± 0.7	10	0
Farrerdale,	ketoprofen	15	14 (93)	1.5 ± 1.4	14	2 (14)
SK	saline	15	12 (80)	1.3 ± 1.3	13	1 (8)
Jumping	ketoprofen	8	8 (100)	1.5 ± 0.9	15	2 (13)
Deer Creek, SK	saline	10	9 (90)	1.7 ± 1.0	16	2 (13)
Minnedosa,	ketoprofen	12	12 (100)	2.2 ± 1.3	17	3 (18)
MB	saline	12	12 (100)	2.0 ± 1.4	17	3 (18)
All sites	ketoprofen	45	42 (93)	1.9 ± 1.2	57	8 (14)
	saline	43	37 (88)	1.7 ± 1.2	56	6 (11)

Table 7.2. Nest initiation and days devoted to egg laying and incubation (mean ± SD) for female mallards at four sites in prairie Canada following surgery to implant an intra-abdominal radio transmitter during isoflurane-anesthesia with either ketoprofen or saline given intramuscularly.

Study Area	Treatment	n	Nest Initiation		Days Devoted
			Median Date[a]	Days After Surgery	
Donalda, AB	ketoprofen	11	134 (109 - 145)	32 ± 11	16 ± 11
	saline	7	136 (120 - 157)	36 ± 13	8 ± 6
Farrerdale, SK	ketoprofen	15	123 (111 - 147)	27 ± 11	10 ± 11
	saline	15	124 (110 - 149)	27 ± 11	12 ± 10
Jumping Deer Creek, SK	ketoprofen	13	124 (112 - 138)	23 ± 9	11 ± 8
	saline	15	124 (112 - 141)	24 ± 10	14 ± 10
Minnedosa, MB	ketoprofen	13	124 (108 - 163)	31 ± 17	15 ± 9
	saline	17	132 (109 - 165)	35 ± 17	15 ± 12
All sites	ketoprofen	51	124 (108 - 163)	27 ± 13	12 ± 1.6
	saline	55	125 (109 - 165)	29 ± 14	13 ± 1.7

[a]median Julian date for each female's first nest (range)

Table 7.3. Hen mortality (%) and number of days surviving following surgery (mean ± SD) to implant an intra-abdominal radio transmitter during isoflurane-anesthesia with either ketoprofen or saline given intramuscularly in female mallards at four sites, in prairie Canada.

Study Area	Treatment	n	Hen Mortality	n	Days Survived
Donalda, AB	ketoprofen	19	3 (16)	3	52 ± 22
	saline	20	6 (30)	6	38 ± 8
Farrerdale, SK	ketoprofen	20	5 (25)	5	35 ± 13
	saline	20	4 (20)	4	29 ± 15
Jumping Deer	ketoprofen	20	1 (5)	1	18
Creek, SK	saline	20	1 (5)	1	26
Minnedosa,	ketoprofen	20	5 (25)	5	56 ± 16
MB	saline	20	2 (10)	2	49 ± 2
All sites	ketoprofen	79	15	15	40 ± 19
	saline	80	13	13	34 ± 12

8. ASSESSMENT OF KETOPROFEN AND BUPIVACAINE FOR ANALGESIA IN FEMALE NESTING MALLARD DUCKS FOLLOWING SURGICAL PLACEMENT OF INTRA-ABDOMINAL TRANSMITTERS

8.1 Introduction

Wildlife managers rely on information obtained from radio telemetry studies for their management decisions. Transmitters are used to provide valuable information on reproduction, movement patterns, habitat use, and survival in a variety of wildlife species, including waterfowl. Intra-abdominal transmitters are often used in waterfowl preferentially over externally-mounted transmitters (Korschgen et al. 1984) as they appear to have less effect on normal behaviour (Greenwood and Sargeant 1973; Pietz et al. 1993) and reproductive effort (Paquette et al. 1997; Pietz et al. 1993; Rotella et al. 1993). In most brood survival studies, females have been equipped with transmitters during mid-to late incubation to reduce loss of equipment and information due to predation or the possibility of nest abandonment (Gloutney et al. 1993). Placement of intra-abdominal transmitters necessitates an incision in the brood patch which may have significant implications, such as altered incubation patterns resulting in delayed hatch, for females that are implanted during incubation.

Birds and mammals likely experience pain in a similar manner. Birds possess the neurological components to respond appropriately to painful stimuli (Jones et al. 1985; Willis et al. 1979), have endogenous antinociceptive mechanisms to modulate pain (Bayon et al. 1980; Csillag et al. 1989; Reiner et al. 1984), and treatment with pharmacological agents used in mammals modulates pain pathways and behavioural responses to painful stimuli (e.g. (Curro et al. 1994; Danbury et al. 1997; Glatz et al. 1992; Paul-Murphy et al. 1999). Modification of normal behaviour may result in erroneous interpretation of data if unrecognized subtle changes occur in the physiology, behaviour or welfare of the bird (Houston and Greenwood 1993). As pain is produced by any procedure or injury that causes tissue damage (Kanjhan 1995), it is likely that waterfowl implanted with radio transmitters would experience pain.

Analgesia can be defined as the relief of pain without loss of consciousness. Analgesics function by decreasing the stimulation of ascending spinal pathways or by activating endogenous descending pain modulation pathways (Clyde 1994). Analgesics, such as opioids (e.g. morphine) or alpha$_2$-agonists (e.g. xylazine) could be used to provide analgesia but residual sedation (Plumb 1995) may preclude their use in free-ranging waterfowl due to compromised survival. Local anesthetics and nonsteroidal anti-inflammatory drugs (NSAIDs) do not have sedative effects (Plumb 1995) making NSAIDS potentially more useful in providing operative and post-operative analgesia to free-ranging waterfowl undergoing implantation of radio transmitters.

Local anesthetics, such as lidocaine and bupivacaine, function by blocking ion channels thereby preventing impulse conduction of pain (Courtney 1980). In avian

species, local anesthetics have been used for operative and/or post-operative pain relief (Paul-Murphy and Ludders 2001). In domestic chickens, bupivacaine has produced effective analgesia in pain models (Glatz et al. 1992; Hocking et al. 1997). Bupivacaine has been also used to provide analgesia following intra-abdominal transmitter implant but long-lasting analgesia was likely not achieved in free-ranging ruddy ducks (Chapter 6).

Non-steroidal anti-inflammatory drugs may provide longer lasting analgesia compared with local anesthetics because they decrease inflammation associated with the surgery and through direct central nervous system effects (Dirig and Yaksh 1997; Mathews 2000). Nonsteroidal anti-inflammatory drugs control pain by inhibiting the enzyme cyclooxygenase which, in turn, prevents the production of prostaglandins (PGs) (Mathews 2000). PGs are involved in the modulation of avian pain responses and the physiological mechanisms involving avian PGs are similar to mammalians (Nicol et al. 1992). Ketoprofen has been used to provide analgesia in many mammalian species (Mathews 2000) and may exert pharmacological and/or physiological actions for up to 12 hours in mallard ducks (Machin et al. 2001, Chapter 3).

Surgically induced stress responses are evoked by nociceptive afferent activity induced by tissue damage and manipulation, even in patients that are receiving adequate general anesthesia (Benson et al. 2000). Analgesics decrease the stress response and are most effective when administered pre-emptively (Woolf and Chong 1993). Attenuation of the stress response through adequate pain relief may result in improved healing and patient outcome (Benson et al. 2000). Neuroendocrine assays have been used in an

attempt to identify an indication of pain (Smith et al. 1999). In avian species, corticosterone is the principal stress corticosteroid in birds (Holmes and Phillips 1976) but progesterone has also been identified as an indicator of stress in avian species (Al-Ankari 1998; Gratto-Trevor et al. 1991).

The objective of this study was to determine the effectiveness of ketoprofen and bupivacaine analgesia by comparing pre- and post- operative incubation patterns, length of post-operative incubation breaks, length of time required to warm eggs, and days from surgery to hatch. To determine the impact of a stressor, the surgical implantation of a radio transmitter into the abdomen of a female mallard during incubation, incubation patterns were examined. In addition, the role of analgesia in the perioperative stress response were examined by measuring plasma corticosterone and progesterone levels.

8.2 Materials and Methods

All ducks were treated in accordance with guidelines of the Canadian Council on Animal Care as defined by the Guide to the Care and Use of Experimental Animals and was approved by the University of Saskatchewan Animal Care Committee. Work was conducted from May to July 1997 and 1998. Wild strain (F1; hatched in captivity from eggs taken from the wild, and F2) captive mallards were held in an outdoor facility at the St. Denis National Wildlife Area, Saskatchewan (52° 20' N, 106° 10' W). Twenty six nesting female mallard ducks (*Anas platyrhynchos*) were used in this study and were provided with unrestricted access to food and water. Seventeen females were housed

individually in pens measuring 3 x 10 m with 30% water and 70 % dry land, the latter consisting primarily of brome grass and shelter provided was a plywood box. The remainder of the females were housed in a 20 X 30 m pen that also held non-reproductive females and males with access to shelter, water and dry land, as described above.

All ducks were anesthetized with propofol. Propofol was delivered through an intravenous catheter placed in the medial metatarsal vein as previously described by Machin and Caulkett (2000). Induction of anesthesia was accomplished by administering 10 mg/kg of propofol as a loading dose and anesthesia was maintained by administering additional boluses of 1 to 2 mg/kg as needed throughout the procedure to maintain a constant level of anesthesia (Table 1). Depth of anesthesia was assessed by monitoring (a) heart rate using an esophageal stethoscope, (b) nictitating membrane movements, (c) swallowing or coughing, (d) response to stimuli and (e) movement. All ducks were intubated with a non-cuffed endotracheal tube (3.0 to 3.5 mm, Mallinkrodt Medical Inc., St Louis, MO, 63042, USA) during the surgery and were ventilated using a pediatric self-inflating resuscitation apparatus (AMBU bag). Ventilation was performed by giving one breath per 5 seconds and ensuring expansion of the thorax was visible. After surgery, females were returned to the nest and given additional propofol (2.5 to 1.2 mg/kg) until depth of anesthesia was sufficient to allow removal of the catheter. Respiratory rate was monitored and any bleeding was stopped by applying Blood Stop Powder (Dominion Veterinary Laboratories LTD., Winnipeg, Manitoba, Canada) and manual pressure.

Blood samples were taken from the jugular vein prior to surgery, immediately after surgery, at 3 days after surgery and at hatch. Samples were taken in the morning within 4 h of each other and they were obtained as quickly as possible (less than 2 min) to avoid stress. Blood samples of 1 to 3 mL were drawn, placed in an integrated plasma separation tube with lithium heparin (Sherwood Davis and Geck Medical, St Louis, MO) and kept in ice water until they could be centrifuged. Samples were centrifuged at 2000 g for 10 minutes and samples were then frozen at -20 °C until analysis.

Duplicate samples from each time point from individual ducks were analysed for corticosterone using a Double Antibody Rat Corticosterone Kit (ICN Pharmaceuticals Inc., Orangeburg, NY, 10962) (Sorenson et al. 1997). Analysis was done according to the instructions provided by the manufacturer. Progesterone was analysed using a radioimmunoassay as described by Rawlings et al. (1984). The intra-assay coefficient of variation for this study was 18.2 ± 2.5 pg/mL (mean ± standard deviation) for corticosterone and 1.5 ± 0.9 ng/mL for progesterone.

The incubation period is the number of days from the onset of incubation to hatch. Monitoring of nest attendance was accomplished by recording nest temperature every 4.8 minutes from 1 day prior to surgery and after surgery until hatch using HOBO XT Temperature Data Loggers (Onset Instruments Corp., Pocasset, MA, USA) attached to a thermistor implanted into a hollow dummy egg. The dummy egg was anchored to a metal rod measuring at least 15 cm and placed in the centre of the clutch. In addition, nest attendance in seven females was also measured with the use of electronic scales (Gloutney 1996) which recorded nest attendance every minute. Data from scales were

used to validate HOBO data. Electronic scale and temperature data were scrutinized visually in graphic and spread sheet form.

Using HOBO data in conjunction with electronic scale data, a decrease of at least 1.5 °C was used as an indication that a female was absent from the nest and a rise in temperature of at least 1.9 °C was indicative of a return to the nest. Breaks were as short as 4 minutes and as long as 91 min (not including the post-operative break). Time spent off the nest of ≤ 3 min were not included as incubation breaks as conceivably this could represent comfort behaviour or adjustment of nesting material. After placement of the electronic scale or dummy egg containing the thermistor, females had several brief (< 3 min) breaks, presumably to rearrange eggs or nesting material. Therefore, these were not included in the analysis of incubation recesses. Only data from the 24 hours immediately preceding the surgery were used for analysis to allow habituation to the scales and dummy eggs for ≥ 36 hours. Questionable data (not easily interpreted from either graph or spread sheets) were excluded from analysis.

Data are reported as mean ± standard deviation. Time spent off the nest, number of recesses, heart rate, amount of propofol required to maintain anesthesia, and plasma corticosterone and progesterone were analysed using a repeated measures analysis of variance (ANOVA). Time spent off the nest and post-operative behavioural data were log transformed to meet normality assumptions of parametric tests. A general linear model (GLM) was used to determine if there were differences among analgesics in the time to first incubation break and length of the first incubation break following surgery, and time required to rewarm the eggs. As clutch size and weather may also influence

incubation patterns, length of time to rewarm eggs, length of incubation break, and length of incubation period were included in initial analyses. However, these variables were removed from further analysis as they were not associated with the incubation patterns analysed. Pearson's correlation was used to determine the association between corticosterone and progesterone immediately after surgery

8.3 Results

Bupivacaine was administered 4.5 ± 2.4 minutes and ketoprofen 6.4 ± 4.1 minutes prior to surgery. Heart rate declined significantly over time (repeated measures ANOVA $F = 50.43$, $df = 3, 72$, $P = 0.001$), but there was no difference between treatments (ketoprofen and bupivacaine, repeated measures ANOVA $F = 0.46$, $df = 1,24$, $P = 0.50$; Figure 8.1). Similarly, no difference between treatments was detected in propofol required for anesthesia during surgery (repeated measures ANOVA $F = 0.22$, $df = 1,24$, $P = 0.64$) nor amount of total propofol administered ($F = 1.19$, $df = 1,24$, $P = 0.29$; Table 8.1).

Treatment did not have an effect on total time spent off the nest (repeated measures ANOVA $F = 0.09$, $df = 1,21$, $P = 0.76$) or number of breaks taken following surgery (repeated measures ANOVA $F = 0.95$, $df = 1,21$, $P = 0.34$). However, for both drugs, the amount of time spent off the nest varied (repeated measures ANOVA $F = 3.04$, $df = 4,84$, $P = 0.02$), with females spending more time off the nest in the 24 hours following surgery compared to prior to surgery and after the 24 hour period following

surgery. None of the other time periods were different nor was there a difference in the number of breaks (Figure 8.2).

No difference between treatments was detected in the time to first incubation break ($F = 0.07$, $df = 1,18$, $P = 0.80$), length of first incubation break following surgery ($F = 0.35$, $df = 1,23$, $P = 0.56$) with ketoprofen-treated females taking a slightly longer break (151.8 ± 133.3) compared to bupivacaine-treated females (98.6 ± 126.3 min), and time required to rewarm the eggs ($F = 2.56$, $df = 1,17$, $P = 0.13$) with bupivacaine-treated females taking slightly longer (122.2 ± 59.8 min) compared with ketoprofen-treated females (90.1 ± 40.8; Figure 8.3). Although not significant, ketoprofen-treated females incubated longer (26.9 ± 47.4 min) immediately following surgery and placement back onto the nest before taking a post-operative break compared with bupivacaine-treated females (15.8 ± 14.6 min). A difference between treatments in numbers of days from surgery to hatch was detected ($F = 4.20$, $df = 1,24$, $P = 0.05$) with bupivacaine-treated females taking 6.2 ± 0.7 days compared to those that received ketoprofen (5.6 ± 0.5 days; Figure 8.4).

Initial blood samples were collected within 80 seconds of capture. Plasma corticosterone and progesterone concentration did not differ between treatments (repeated measures ANOVA $F = 0.03$, $df = 1,12$, $P = 0.86$ and $F = 0.01$, $df = 1,12$, $P = 0.94$, respectively) but differed with among time periods (repeated measures ANOVA $F = 30.25$, $df = 3,36$, $P = 0.0001$ and $F = 19.25$, $df = 3,36$, $P = 0.0001$, respectively). Plasma corticosterone and progesterone concentrations were elevated following surgery compared to the initial and day 3 sample periods (Figure 8.5)

No relationship was found between treatment, corticosterone or progesterone and time to first incubation break ($F = 0.43$, $df = 2,16$, $P = 0.66$) and length of the first incubation break following surgery ($F = 0.83$, $df = 2,20$, $P = 0.45$), time spent away from the nest in the first 24 hours following surgery ($F = 0.18$, $df = 2,20$, $P = 0.13$), or number of breaks taken in the first 24 hours following surgery ($F = 0.50$, $df = 2,20$, $P = 0.61$). However, corticosterone and progesterone were highly correlated $r = 0.83$, $n = 23$, $P = 0.0001$) immediately following surgery (Figure 8.6). Corticosterone concentration was positively correlated with time taken to obtain the sample $r^2 = 0.15$, $df = 1,23$, $P = 0.05$).

Electronic scales were used to validate HOBO data. Meade (1996) and Yerkes (1998) used a rise or drop in temperature of 3 °C was considered an "on" or "off" movement of the female and if the drop in 3 °C was not maintained for longer than 14.4 minutes the hen was considered on the nest and involved in a comfort movement. A drop of 1.5 °C indicative of an "off" movement and arise of at least 1.9 °C was indicative of a return to the nest. Scale data registered a reading every minute compared to the 4.8 minutes by the HOBO temperature logger, thus an error of 9.6 minutes was possible and evident when comparisons of the data were made. In addition, 26 % of the incubation breaks were less than 14.4 minutes in duration, contributing to error in estimating incubation constancy when HOBO temperature loggers are used.

8.4 Discussion

Females received either ketoprofen or bupivacaine for operative and post-operative pain relief for laparotomy to place an intra-abdominal radio transmitter. Ketoprofen and bupivacaine likely provided some analgesia but changed the incubation patterns occurred, as evident by the increased time spent off the nest within the first 24 hrs after surgery. Although statistical differences between groups in relation to incubation patterns were not detected, possibly because power to detect differences was low because of small sample size. Difference between treatments in the number of days following surgery to hatch, suggests that quality of analgesia provided by ketoprofen or bupivacaine may not be equal. Females that received bupivacaine took longer to hatch their brood compared to ketoprofen-treated females even after controlling for clutch size, weather and stage of incubation when surgery occurred. This suggests that the subtle (non-significant) changes in incubation patterns may have been biologically important.

Ketoprofen (NSAID) provides analgesia by decreasing inflammation at the site of injury and also through central nervous system effects (Dirig and Yaksh 1997; Mathews 2000), whereas, bupivacaine (local anesthetic) acts by blocking ion channels thereby preventing impulse conduction of pain (Courtney 1980) from the infiltration site. Ketoprofen may be more effective post-operatively because of its relatively longer action of at least 12 hours (Machin et al. 2001, Chapter 3) compared to bupivacaine infiltration with an action of 2.5 to 6 hours in mammals (Skarda 1996). Therefore, females that received bupivacaine may not have been as effective at incubation following surgery

because of pain in the region of the brood patch. Regulation of internal egg temperature is accomplished by varying the amount of time spent on the nest and the amount of heat transferred to eggs when on the nest (Drent 1975), while heat transfer is regulated by varying the frequency and degree to which the brood patch contacts the eggs (White and Kinney 1974) and by varying blood flow through the brood patch (Haftorn and Reinertsen 1982). Anesthesia of the brood patch may have prevented adequate detection of egg temperature, causeing a decline in circulating prolactin concentrations (Hall 1987), thus resulting in prolonged rewarming of the eggs. However, actual egg temperature was not estimated in this study and although time spent incubating was similar, true egg temperature may have been different. Egg incubation temperatures are an important determinant of embryonic growth and development rates and thus have a significant effect on incubation length (Deeming and Ferguson 1993) which may explain why length of incubation was extended in females that received bupivacaine.

Ketoprofen or bupivacaine administration was < 5 min prior to surgery, and therefore, it is possible that analgesia may not have been fully in effect at the start of surgery. Tissue injury and acute pain, can effect both peripheral and central nervous systems in mammals and birds, and alter sensitivity to subsequent stimuli. Pain-induced neural changes can be prevented by administration of analgesic agents before development of injury induces spinal hyperexcitability and pain related behaviours (Coderre et al. 1990; Katz et al. 1992). However, because of the close proximity of analgesic administration and surgery it is possible that some pain-induced neural changes were not prevented, resulting in disruption of normal incubation in the 24 hour period

following tissue damage for both treatments. The long post-operative incubation break may be representative of discomfort in the region of the brood patch.

Although in other studies, propofol preserved heart rate in mallards when adequate ventilation was provided (Machin and Caulkett 1998), a significant decrease in heart rate was observed in this study . This decline in heart rate may have been the result of an artificially elevated heart rate prior to induction from excitement, or apnea on induction resulting in hypoxia and tachycardia (Taylor et al. 1986). An increase in handling time during acquisition of the first blood sample was correlated with elevated plasma corticosterone levels, indicating stress increased with prolonged handling. Anesthesia began several minutes after capture and initial blood sampling, therefore stress associated with handling was also likely responsible for elevated heart rates (Cabanac and Aizawa 2000). However, anesthetic induction can be stressful (Wilmore et al. 1989) which may further contribute to elevated heart rates initially.

Corticosterone levels were low prior to surgery but collection of samples occurred within a few minutes of capture and likely did not illustrate true level of stress at start of surgery. Interestingly, elevated levels of corticosterone and progesterone at hatch may indicate that this period may also be stressful, and deserves further investigation. Corticosterone and progesterone were elevated and highly correlated following surgery. High levels of corticosterone and progesterone have been associated with a stress response in nesting birds (Gratto-Trevor et al. 1991). Progesterone is the precursor to corticosterone (Carsia and Harvey 2000) and may increase in response to stress (Gratto-Trevor et al. 1991), suggesting that progesterone may be an alternative

bioindicator of stress.

Increases in corticosterone and progesterone can indicate stress (Gratto-Trevor et al. 1991) and/or pain (Lin et al. 1993). Surgery during incubation, altered incubation patterns in the 24 h period following surgery, regardless of analgesic. However, ketoprofen and bupivacaine likely provided analgesia and should be administered prior to surgery. As length of the incubation period was extended in bupivacaine-treated females compared to ketoprofen-treated females, analgesia that interferes with brood patch sensation is likely not desirable.

Care should be taken in interpreting incubation data obtained with the use of HOBO temperature loggers. A drop of 1.5 °C indicative of an "off" movement and a rise of at least 1.9 °C was indicative of a return to the nest was better at estimating incubation behaviour than the 3 °C used by Meade (1996) and Yerkes (1998). Also females may take short incubation breaks (<14.4 min), most of which were detected using smaller changes in temperatures (of 1.5 and 1.9 °C).

Table 8.1. Quantity of propofol (mg/kg, mean ± SD) administered intravenously during anesthesia of female mallards in late incubation for surgical placement of intra-abdominal transmitters with i.m. ketoprofen (5 mg/kg) or bupivacaine infiltration (2 mg/kg) administered prior to surgery.

Treatment	n	Time interval (min)				Total
		0 - 5	5 - 10	10 - 15	15 - 20	
Bupivacaine	13	14.7 ± 2.3	8.8 ± 3.3	5.3 ± 3.0	3.2 ± 4.2	33.1 ± 7.9
Ketoprofen	13	13.0 ± 1.6	8.0 ± 3.9	5.8 ± 2.1	2.5 ± 2.3	30.7 ± 4.6

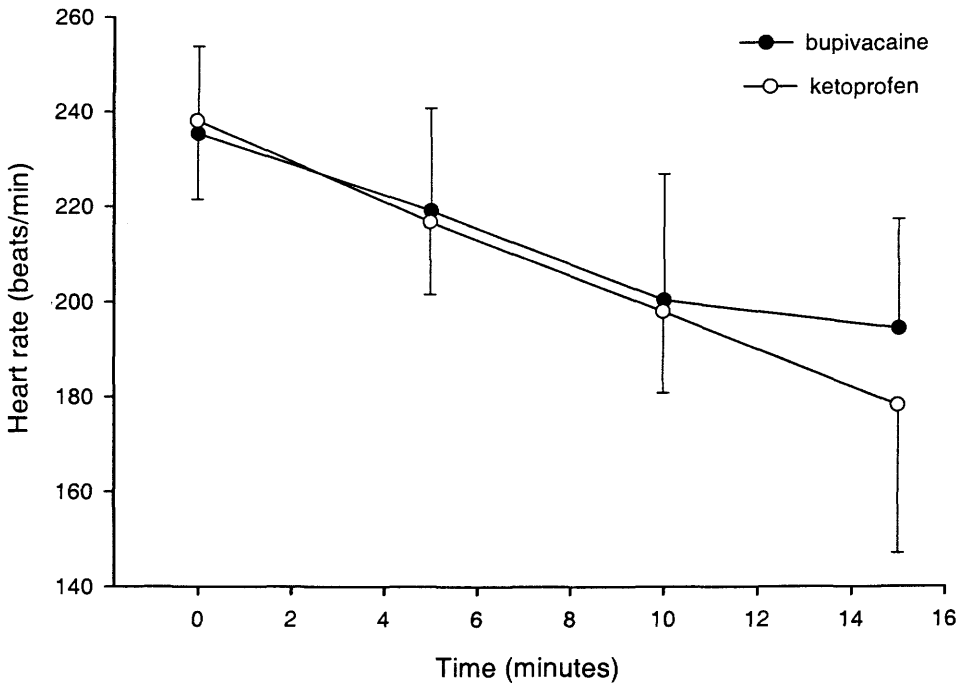

Figure 8.1. Heart rates (mean ± SD) after induction (0 minutes) and during surgery for intra-abdominal transmitter placement during propofol anesthesia with i.m. ketoprofen (5 mg/kg) or bupivacaine infiltration (2 mg/kg) administered prior to surgery.

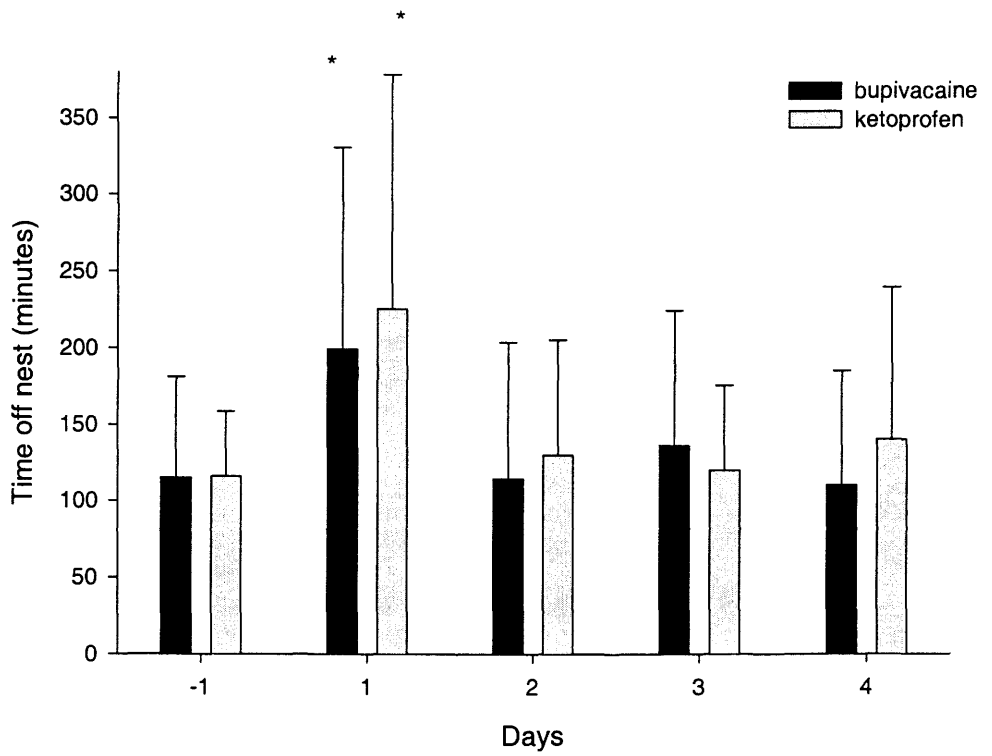

Figure 8.2. Average (± SD) minutes spent off the nest each 24 hour period prior to (-1 days) and following surgery for intra-abdominal transmitter placement during propofol anesthesia with i.m. ketoprofen (5 mg/kg) or bupivacaine infiltration (2 mg/kg) administered prior to surgery. An asterix (*) indicates a significant difference from presurgical values ($P \leq 0.05$)

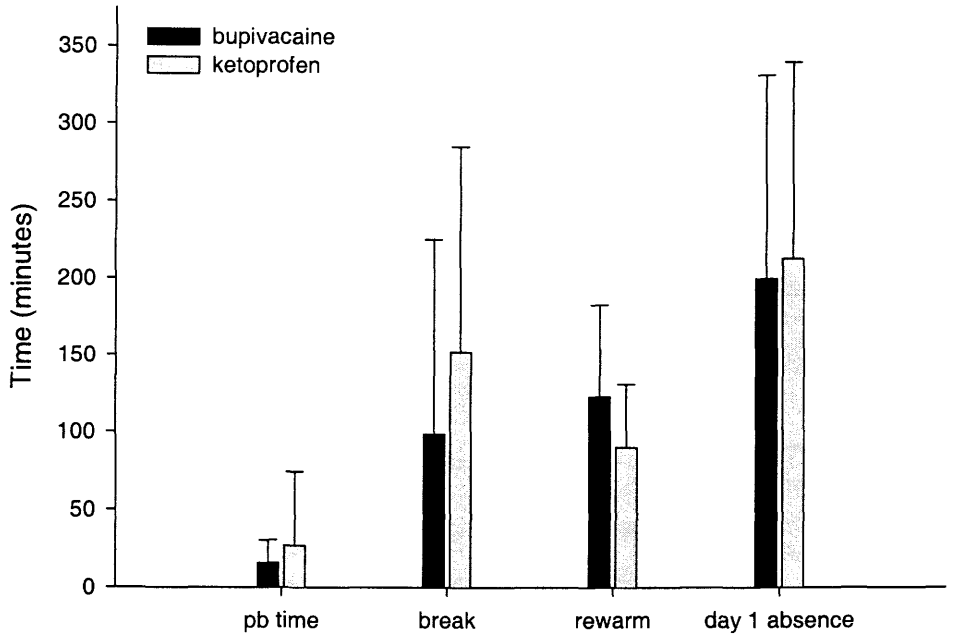

Figure 8.3. Comparison of postoperative incubation behaviour (mean ± SD) of nesting female mallards following intra-abdominal transmitter placement during propofol anesthesia with i.m. ketoprofen (5 mg/kg) or bupivacaine infiltration (2 mg/kg) administered prior to surgery. Post-operative incubation break time (pb time) represents the length of time before the female took a recess following surgery and post-operative break (break) is the length of the first recess immediately following surgery. Rewarm illustrates the time it took for the female to warm eggs to maximum temperature following the recess. Day 1 absence is the amount of time the female was absent from the nest in the first 24 hours following surgery.

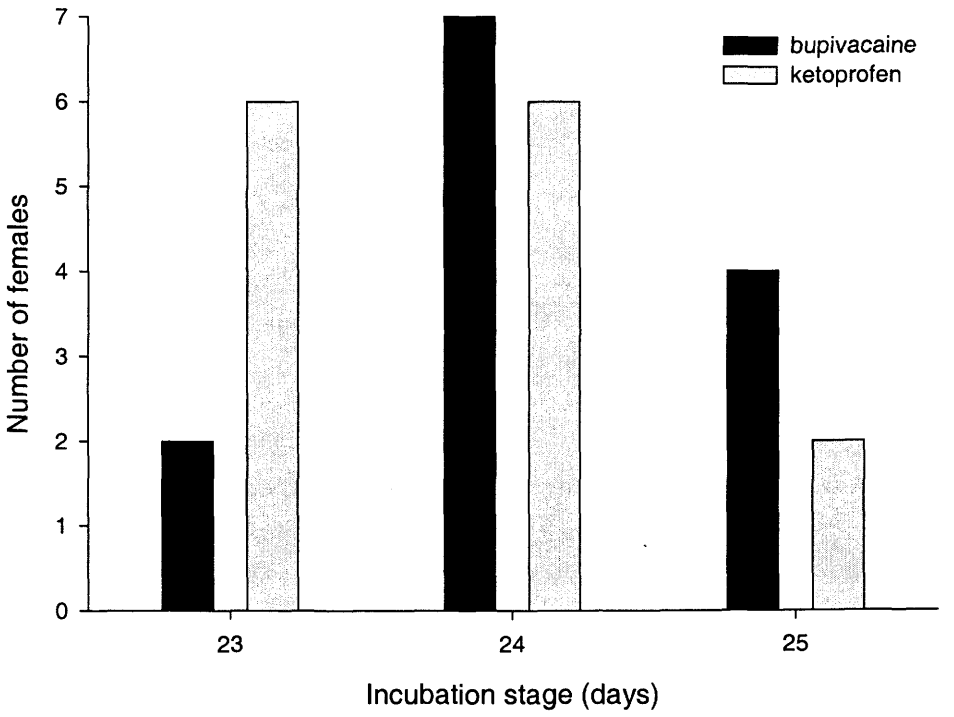

Figure 8.4. Incubation periods of captive female mallards after intra-abdominal transmitter placement during propofol anesthesia with either i.m. ketoprofen (5 mg/kg) or bupivacaine infiltration (2 mg/kg) given prior to surgery.

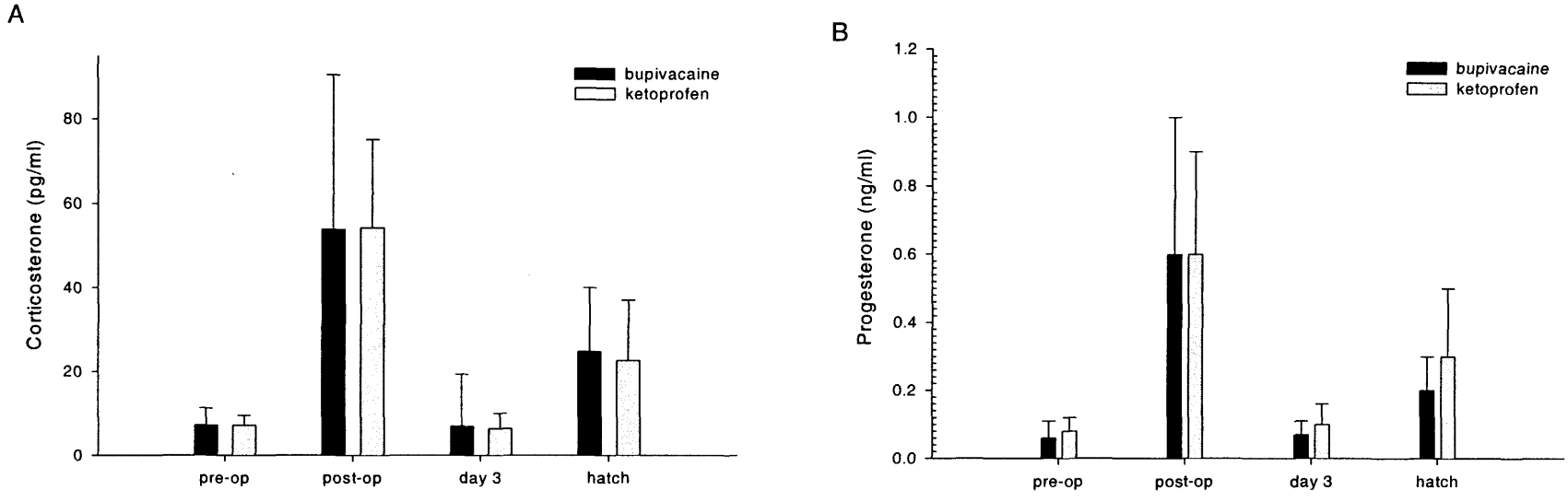

Figure 8.5. Plasma concentration of A) corticosterone (pg/mL) and B) progesterone (ng/mL) prior to (pre-op) and following (post-op) surgery, 3 days after surgery, and at hatch. An intra-abdominal dummy transmitter was placed during propofol anesthesia with i.m. ketoprofen (5 mg/kg) or bupivacaine infiltration (2 mg/kg) administered prior to surgery.

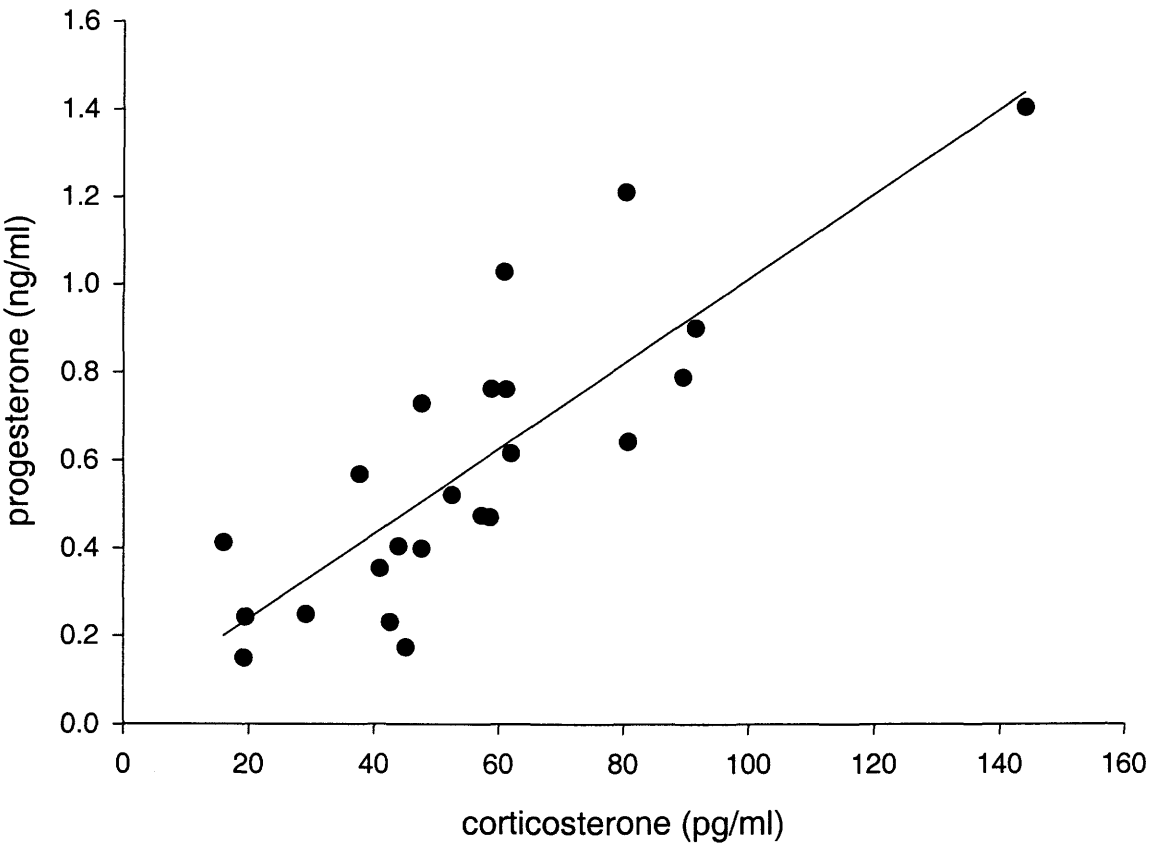

Figure 8.6. Plasma progesterone (ng/mL) vs corticosterone (pg/mL) concentration immediately following surgery for intra-abdominal transmitter placement during propofol anesthesia with i.m. ketoprofen (5 mg/kg) or bupivacaine infiltration (2 mg/kg) administered prior to surgery. Pearson's Correlation $r = 0.83$, $n = 23$, $P = 0.0001$.

9. GENERAL DISCUSSION AND CONCLUSIONS

Many field and laboratory studies use various forms of manipulation (e.g. capture, handling, blood sampling, radio transmitter attachment) to gain more insight into animal ecology and behaviour with little attention to the ethical and conservation implications of such wildlife manipulations (Putman 1995). It is generally accepted that birds perceive pain similar to mammals. Birds possess the neurological components to respond appropriately to a painful stimulus (Jones et al. 1985; Willis et al. 1979), have endogenous antinociceptive mechanisms to modulate pain (Bayon et al. 1980; Csillag et al. 1989; Reiner et al. 1984), and treatment with pharmacological agents used in mammals modulates pain pathways and behavioural responses to painful stimuli (e.g. (Curro et al. 1994; Danbury et al. 1997; Glatz et al. 1992; Paul-Murphy et al. 1999)). However, birds often do not indicate pain in an obvious manner because species that may be preyed upon are less likely to display overt pain-associated behaviour that may attract attention of predators (Livingston 1994). Considerable variation in behavioural responses to pain may occur among avian species, breeds, strains, or individuals and there is no reliable or universal indicator of pain (Gentle 1992). As pain can be produced by any procedure or injury that causes tissue damage, waterfowl implanted with radio transmitters likely experience pain. No study has specifically addressed the role of pain during and after the placement of intra-abdominal radio transmitters.

Timely administration of analgesics is important as ongoing pain perception can have a negative effect on homeostasis and healing (Benson et al. 2000). Opioids (e.g. morphine) or alpha$_2$-agonists (e.g. xylazine) could be used to provide analgesia but residual sedation (Plumb 1995) may preclude their use in free-ranging waterfowl. The sedative effects can be reversed with drugs antagonizing the effects but analgesia would also be discontinued (Martin 1984). Therefore, this study concentrated on nonsteroidal anti-inflammatory drugs (NSAIDs) and local anesthetics which do not have sedative effects (Plumb 1995).

This study attempted to gain insight into the mechanisms and sublethal effects of pain and stress by evaluating several physiological, behavioural and reproductive indices of fitness. More specifically, the role of analgesia in alleviating pain during and following transmitter placement was investigated. An investigation of the pharmacodynamics of nonsteroidal anti-inflammatory drugs (flunixin and ketoprofen) and the pharmacokinetics of a local anesthetic (bupivacaine) provided insight into how these agents function in an avian model. The role of stress during pain was also explored by measuring plasma levels of corticosterone and progesterone prior to and following surgery.

An investigation of the pharmacological action of two nonsteroidal anti-inflammatory drugs (flunixin and ketoprofen) was undertaken by measuring thromboxane B_2. Flunixin and ketoprofen may exert pharmacological effects for at least 12 hours. However, the use of flunixin in waterfowl cannot be recommended as it produced extensive muscular necrosis which may, in itself, produce pain. Single

administration of flunixin and ketoprofen was not associated with renal changes but the safety of the repetitive use of these drugs on renal function in ducks needs to be determined. Stress may play a role in plasma thromboxane levels.

Mallard ducks anesthetized with isoflurane demonstrated response to painful stimuli with an increase in heart and respiratory rates. Ketoprofen produced a significant analgesic effect which was measured as a reduced response in heart and respiratory rates compared to saline controls. However, administration of ketoprofen, earlier than 30 minutes prior to surgery, may be necessary to ensure that ketoprofen's analgesic effects are present in 100 % of the animals.

An examination of the effects of analgesia on reproductive fitness of free-ranging mallards was made by comparing females that received ketoprofen prior to surgery with those that did not (saline controls). A significant surgeon effect was detected, in that, longer surgeries were correlated with increased time to first nesting attempt. Females that received ketoprofen took 3.5 days less to first nest attempt than did females that received saline, indicating that analgesia was beneficial, and there was no evidence to suggest that ketoprofen was harmful. However, ketoprofen may have more benefit if administered well in advance of surgery, to allow time for the drug to exert its analgesic effects.

An investigation measuring plasma levels of bupivacaine indicated that it may be shorter acting in ducks than in mammals, however, it is difficult to make comparisons between studies with differing methodology. Sequestration and redistribution of bupivacaine may result in a delayed toxicity but mechanisms are unknown. A shorter

absorption time compared to elimination time may, in part, explain avian sensitivity to local anesthetics, however, more information on drug distribution is required to draw concrete conclusions. Pharmacokinetics may contribute to the sensitivity of avian species to local anesthetics but other possible mechanisms could be involved and more studies are necessary to elucidate the mechanism of local anesthetic toxicity in avian species.

A study examining the post-operative behaviour of male and female ruddy ducks determined that bupivacaine does not appear to achieve long-term analgesia or prevention of post-operative pain-related behaviours. However, it does not preclude the possibility that there was some short-term or pre-emptive benefit. Increasing bupivacaine dose to attain longer lasting pain relief would not be beneficial as birds may be more sensitive to the toxic effects of local anesthetics compared to mammals, as lower doses i birds (2.7 - 3.3 mg/kg) (Hocking et al. 1997) produce toxic effects compare with higher doses (3.5 -4.5 mg/kg) in dogs (Skarda 1996).

A comparison of ketoprofen and bupivacaine analgesia was compared in incubating female mallard ducks implanted with dummy radio transmitters at 18 days of incubation. Surgery altered incubation patterns in the 24 h period following surgery, regardless of analgesic. Length of the incubation period was extended in bupivacaine-treated females compared to ketoprofen-treated females, indicating that analgesia may interfere with brood patch sensation. However, ketoprofen and bupivacaine likely provided analgesia but the parameters monitored were not the best indicators of post-operative analgesia. Increases in corticosterone and progesterone were detected following surgery which may indicate stress (Gratto-Trevor et al. 1991) and/or pain (Lin

et al. 1993).

The benefits of administering analgesia cannot be overlooked in minimizing effects of placement of radio transmitters on free-ranging wildlife. This study clearly demonstrates that there are benefits but also that more research is necessary to determine the impacts of intervention in field experiments. Handling techniques, alone, are known to cause physiological responses, particularly in nonacclimated animals (Gartner et al. 1980) that may bias or invalidate results of studies. A study using captive domestic geese demonstrated that weighing, injecting, and blood sampling disrupted the acid-base balance and caused a dramatic increase in the level of humoral indices of stress (catecholamines, corticosterone and lactate) within 2 minutes (Le Maho et al. 1992). However, the long-term effects of handling and other manipulations are largely unknown.

Declines in duck populations have led to listing or anticipated listing some species as "threatened" in Canada or the United States. The cause of decreased population numbers are varied and not always known. Low recruitment and low survival during the past two decades may be partly responsible for declines in many species. Recruitment is influenced by: 1) habitat conditions and weather patterns on breeding, migration, and wintering areas, 2) physiological condition of the birds, 3) age structure of the population, 4) predation on eggs, females, and broods, and 5) population density. Relatively dry breeding habitat conditions, intensified agricultural production, and long term changes in the abundance and composition of predators are mainly responsible for low numbers in many species. Physiological condition is influenced by

availability of food, sanctuaries, and resting sites and weather conditions in wintering and migration areas (DucksUnlimited 1994). Human impact on wildlife and wildlife habitat continues to increase, which ultimately increases the stresses that an animal or population experiences. In order to understand the complexity and integration of physiological and behavioural responses to human impacts and intervention it is necessary to measure several variables. Links between molecular processes on a cellular level and the organismal response to stress are becoming increasingly recognized (van Eden and Young 1996). While "biological conservation generally concentrates on populations and species communities and rarely considers mechanism operating at the level of the individual that may be ultimately responsible for population and community changes" (Hofer and East 1998). Future research will be directed at identifying the physiological mechanisms and markers which may elucidate the effect of stress on wildlife.

10. REFERENCES

Aarsman, A.J., Mynbee, G., Van den Bosh, H., Rothhut, B., Prierus, B. and Comera, C. 1987. Lipocortin inhibition of extracellular and intracellular phopholipases A2 is substrate concentration dependent, FEBS Letters, 219 176-180.

Al-Ankari, A.-R.S. 1998. Relationship between gonadal steroids and corticosterone during blood sampling in saker falcons, J Wildl Dis, 34 653-655.

Anderson, M.G., Sayler, R.D. and Afton, A.D. 1980. A decoy trap for diving ducks, J Wildl Manage, 44 217-219.

Apfelbaum, J.L., Grasela, T.H., Hug, C.C.J., McLeskey, C.H., Nahrwold, M.L., Roizen, M.F., Stanley, T.H., Thisted, R.A., Walawander, C.A. and White, P.F. 1993. The initial clinical experience of 1819 physicians in maintaining anesthesia with propofol: characteristics associated with prolonged time to awakening, Anesth Analg, 77 (Suppl 4) S10-14.

Armstrong, D., Dry, R.M.L., Keele, C.A. and Markham, J.W. 1953. Observations on chemical excitants of cutaneous pain in man., J Physiol, 120 326-351.

Arthur, G.R. 1987. Pharmacokinetics of local anesthetics. In: G.R. Strichartz (Ed.), Handbook of Experimental Pharmacology: Local Anesthetics, Vol. 81, Springer-Verlag, Berlin, pp. 165-186.

Arthur, G.R. 1988. Comparative pharmacokinetics of bupivacaine and ropivacaine, a new amide local anesthetic., Anesth Analg, 67 1053-1058.

Bailey, I.A., Carey, F., Haworth, D. and J., S.H. 1983. Effects of vascular trauma and transient myocardial ischaemia on coronary venous prostaglandin levels in the dog, Cardiovasc Res, 17 127-131.

Bakken, G.S., Reynolds, P.S., Kenow, K.P., Korschgen, C.E. and Boysen, A.F. 1996. Thermoregulatory effects of radiotelemetry transmitters on mallard ducklings, J Wildl Manage, 60 669-678.

Bakken, G.S., Reynolds, P.S., Kenow, K.P., Korschgen, C.E. and Boysen, A.F. 1999. Metabolic response to air temperature and wind in day-old mallards and a standard operative temperature scale, Physiol Biochem Zool, 72 656-665.

Bardo, M.T. and Hughes, R.A. 1978. Shock-elicited flight response in chickens as an index of morphine analgesia, Pharmacol Biochem Behav, 9 147-149.

Bayon, A., Koda, L., Battenberg, E., Azad, R. and Bloom, F.E. 1980. Regional distribution of endorphin, met5-enkephalin and leu5-enkephalin in the pigion brain, Neurosci Lett, 16 75-80.

Beitel, R.E. and Dubner, R. 1976. Responses of unmyelinated (C) polymodal nociceptors to thermal stimuli applied to monkey's face., Journal of Neurophysiology, 39 1160-1175.

Bennett, R.A. 1993. Surgical Considerations. In: B.W. Ritchie, G.J. Harrison and L.R. Harrison (Eds.), Avian Medicine: principles and application, wingers Publishing, Inc., Lake Worth, Florida, pp. 1081-1095.

Benson, G.J., Grubb, T.L., Neff-Davis, C., Olson, W.A., Thurmon, J.C., Lindner, D.L., Tranquilli, W.J. and O. Vanio. 2000. Perioperative stress response in the dog: effect of pre-emptive administration of medetomidine, Vet Surg, 29 85-91.

Berde, C.B. and Strichartz, G.R. 2000. Local Anesthetics. In: R.D. Miller (Ed.), Anesthesia, Vol. 1, Churchill Livingstone, Philadelphia, pp. 491-521.

Bessou, P. and Perl, E.R. 1969. Response to cutaneous sensory units with unmyelinated fibres to noxious stimuli., J Neurophysiol, 32 1025-1043.

Blackwell, G.I., M., C., DiRosa, R.J. and al, e. 1980. Macrocortin: a polypeptide causing the anti-phospholipase effect of glucocorticoids, Nature, 187 147-149.

Blumberg, H. and Janig, W. 1984. Discharge pattern of afferent fibres from a neuroma, Pain, 20 335-353.

Breazile, J.E. 1987. Physiologic basis and consequences of distress in animals, J Am Vet Med Assoc, 191 1212-1215.

Breward, J. and Gentle, M.J. 1985. Neuroma formation and abnormal affernt nerve discharges after parial beak amputation (beak trimming) in poultry, Experientia, 41 1132-1134.

Brown, P.W. and Hunter, M.L., Jr. 1985. Potential effects of insecticides on the survival of dabbling duck broods, J Minn Acad Sic, 50 41-45.

Brua, R.B., 1998. Factors affecting the reproductive success of male and female ruddy ducks in southwestern Manitoba, Canada, University of Dayton, Dayton, pp. 134.

Brua, R.B. and Machin, K.L. 2000. Determining and testing the accuracy of incubation stage of ruddy duck eggs by floation, Wildfowl, 51 181-189.

Bruguerolle, B. and Prat, M. 1987. Temporal changes in bupivacaine kinetics, J Pharm Pharmacol, 39 148-149.

Bruguerolle, B., Valli, M., Bouyard, L., Jadot, G. and Bouyard, P. 1983. Effect of the hour of administration on the pharmacokinetics of lidocaine in the rat, Eur J Drug Metab Pharmacokinet, 8 233-238.

Burney, R.G., DiFazio, C.A. and Foster, J.H. 1978. Effects of pH on protein binding of lidocaine, Anesth Analg, 57 478-480.

Bustamante, D., Paeile, C., Willer, J.-C. and Le Bars, D. 1997. Effects of intrathecal or intrcerebroventricular administration of nonsteroidal anti-inflammatory drugs on a c-fiber reflex in rats, Pharmacol Exp Therap, 281 1381-1391.

Butler, P. J. 1988. The exercise response and the "classical" diving response during natural submersion in birds and mammals, Can J Zool, 66 29-39.

Cabanac, M. and Aizawa, S. 2000. Fever and tachycardia in a bird after simple handling, Physiol Behav, 69 541-545.

Cambridge, A.J., Tobias, K.M., Newberry, R.C. and Sarkar, D.K. 2000. Subjective and objective measurements of postoperative pain in cats, J Am Vet Med Assoc, 217 685-690.

Campbell, J.N. and Meyer, R.A. 1983. Sensitization of myelinated nociceptive afferents in monkey varies with skin type, J. Neurophysiol, 49 98-110.

Carsia, R.V. and Harvey, S. 2000. Adrenals. In: G.C. Whittow (Ed.), Sturkie's Avian Physiology, Academic Press, New York, pp. 489-538.

Cashman, J.N. 1996. The mechanisms of action of NSAIDs in analgesia, Drugs, 52 13-23.

Catterall, W. and Mackie, K. 1995. Local Anesthetics. In: J.G.G. Hardman, A. Gilman and L.L. Limbird (Eds.), Goodman & Gilman's the pharmacological basis of therapeutics, McGraw-Hill, New York, pp. 338-347.

Chapman, C.R., Casey, K.L., Dubner, R., Foley, K.M., Gracely, R.H. and Reading, A.E. 1985. Pain Measurement: an Overview, Pain, 22 1-31.

Chen, L. and Huang, L.-Y.M. 1992. Protein kinase C reduces Mg2+ block of NMDA-receptor channels as a mechanism of modulation, Nature, 356 521-523.

Cherng, C.H., Wong, C.S. and Ho, S.T. 1996. Spinal actions of nonsteroidal anti-inflammatory drugs, Acta Anaesthesiol Singapore, 34 i-ii.

Claeys, M.A., Gepts, E. and Camu, F. 1988. Haemodynamic changes during anaesthesia induced and maintained with propofol, Br J Anaesth, 60 3-9.

Clark, L. 1995. Modulation of avian responsiveness to chemical irritants: effects of prostaglandin E1 and analgesics, J Exp Zool, 271 432-440.

Clarke, C.R. 1991. Effect of dexamethazone and phenylbutazone on eicosanoid production in a Pasteurella haemoytica infection model, Acta Vet Scand, S87 257-259.

Clyde, V.L. 1994. Avian analgesia, Proc Am Assoc Zoo Vet, pp. 125-127

Clyde, V.L. and Paul-Murphy, J. 1999. Avian Analgesia. In: M.E. Fowler and R.E. Miller (Eds.), Zoo and Wild Animal Medicine: Current Therapy 4, WB Saunders, Philadelphia, pp. 309-314.

Coderre, T.J., Katz, J., Vaccarino, A.L. and Melzack, R. 1993. Contribution of central neuroplasticity to pathological pain: review of clinical and experimental evidence, Pain, 52 259-285.

Coderre, T.J. and Melzack, R. 1987. Cutaneous hyperalgesia: contributions of the peripheral and central nervous systems to the increase in pain senstivity after injury, Brain Res, 404 95-106.

Coderre, T.J., Vaccarino, A.L. and Melzack, R. 1990. Central nervous system plasticity in the tonic pain response to subcutaneous formalin injection, Brain Res, 535 155-158.

Concannon, K.T., Dodam, J.R. and Hellyer, P.W. 1995. Influence of a mu- and kappa-opioid agonist on isoflurane minimal anesthetic concentration in chickens, Am J Vet Res, 56 806-811.

Cork, S.C., Alley, M.R. and Stockdale, P.H.G. 1995. A quantitative assessment of hemosiderosis in wild and captive birds using image analysis, Av Path, 24 239-254.

Courtney, K. 1980. Structure-activity relations for frequency-dependant sodium channel block in nerve by local anesthetics, J Pharmacol Exp Ther, 213 114-119.

Covino, B.G. and Vassalo, H.G. 1976. Local anesthetics: mechanisms of action and clinical use, Grune & Stratton, New York.

Craig-Schmidt, M.C., Faircloth, S.A. and Weete, J.D. 1986. Modulation of avian lung eicosanoids by dietary omega-3 fatty acids., J Nut, 117 1197-1206.

Csillag, A., Bourne, R.C., Kalman, M., Boxer, M.I. and Stewart, M.G. 1989. [3H]Naloxone binding in the brain of the domestic chick (Gallus domesticus) determined by in vitro quantitative autoradiography, Brain Res, 479 391-396.

Curro, T.G. 1994. Evaluation of the isoflurane-sparing effects of butorphanol and flunixin in psittaciformes, Proc Assoc Av Vet.

Curro, T.G., Brunson, D.B. and Paul-Murphy, J. 1994. Determination of the ED50 of isofluane and evaluation of the isoflurane-sparing effect of butorphanol in cockatoos (Cacatua spp.), Veterinary Anesthesia, 23 429-433.

Danbury, T., Carole, Pain associated with lameness in broiler chickens: a behavioural and pharmacological study, Departments of Pharmacology and Clinical Veterinary Science, Univeristy of Bristol, Bristol, 1999, pp. 234.

Danbury, T.C., Chambers, J.P., Weeks, C.A., Waterman, A.E. and Kestin, S.C. 1997. Self-selection of analgesic drugs by broiler chickens, Animal Choices, 20 126-127.

Danbury, T.C., Hudson, A.L., Waterman-Pearson, A.E., Henderson, G. and Kestin, S.C. 1998. Satuation biniding of u, g, and k opiod ligands in chicken brains, Arch Pharm, 358 (suppl 35) 105.

Danbury, T.C., Ruban, B., Hudson, A.L., Watermane-Pearson, A.E. and Kestin, S.C. 1998. Imadzoline 2 binding sites in chicken brain, Anals N Y Acad Sci, 881 189-192.

Davies, J.A. and Walford, A.J. 1986. Intravenous regional anaesthesia for foot surgery, Acta Anaesthesiol Scand, 30 145-147.

Davis, J.B., Miller, D.L., Kaminski, R.M. and Vrtiska, M.P. 1999. Evaluation of a radio transmitter for wood duck ducklings, J Field Ornith, 70 107-113.

De Grood, P.M.R.M., Ruys, A.H.C., Van Edmond, J., Booij, L.H.D.H. and Crul, J.F. 1985. Propofol ('Diprivan') emulsion for total intravenous anesthesia, Postgrad Med, 61 (Suppl. 3) 65-69.

Deeming, D.C. and Ferguson, M.W.J. 1993. Physiological effects of incubation temperature on embryonic development in reptiles and birds. In: D.C. Deeming and M.W.J. Ferguson (Eds.), Egg incubation: its Effects on Embryonic Development in Birds and Reptiles, Cambridge Univiversity Press, Cambridge.

Dhuner, K.-G. and Lewis, D.H. 1966. Effect of local anesthetics and vasoconstrictors upon regional blood flow, Acta Anaesth Scand, 23 (Suppl) 347 -352.

Dirig, D.M. and Yaksh, T.L. 1997. Spinal and systemic cyclooxygenase inhibitiors supress paw carrageenan-evoked thermal hyperalgesia in rats, Anesthesiology, 87 A721.

Dohoo, S.E. 1990. Isoflurane as an inhalational anesthetic agent in clinical practice, Can Vet J, 31 847-850.

Drent, R.H. 1975. Incubation. In: D.S. Farner and J.R. King (Eds.), Avian biology, Vol. 5, Academic Press, New York, pp. 333-420.

Ducks Unlimited. 1994. Ducks Unlimited Continental conservation plan: an analysis of North American waterfowl populations adn a plan to guide the conservation programs of Ducks Unlimited through the year 2000, pp. 379.

Duke, T. 1995. A new intravenous agent: propofol, Can Vet J, 36 181-182.

Duncan, I.J.H., Beatty, E.R., Hocking, P.M. and Duff, S.R.I. 1991. Assessment of pain associated with degenerative hip disorders in adult male turkeys, Research in Vet Sci, 50 200-203.

Duncan, I.J.H., Slee, G.S., Seawright, E. and Breward, J. 1989. Behavioural consequences of partial beak amputation (beak trimming) in poultry, Br Poult Sci, 30 479-488.

Dzus, E.H. and Clark, R.G. 1997. Overland travel, food abundance, and wetland use by mallards: relationships with offspring survival, Wilson Bull, 109 504-515.

Eccleston, C. 1995. Chronic pain and distraction: An experimental investigation into the role of sustained and shifting attention in processing of chronic persistent pain, Behav Res Therap, 33 391-405.

Englesson, S. and Grevsten, S. 1974. The influence of acid-base changes on central nervous system toxicity of local anaesthetic agents II, Acta Anaesth Scand, 18 88-103.

Eskeland, B. 1981. Effects of beak trimming, First European symposium on poultry welfare. Proc World Poult Sci Assoc, Danish branch, Copenhagen.

Fan, S.G., Shutt, A.J. and Vogt, M. 1981. The importance of 5-hydroxytrptamine turnover for the analgesic effect of morphine in the chicken, Neurosci, 6 2223-2227.

Fisele, J.H., Eger, E.I. and Muallem, M. 1967. Narcotic properties of carbon dioxide in the dogs, Anesthesiology, 27 856-865.

Flecknell, P.A. 1988. The control of pain in animals. In: G.S.G. Grunsell, M.-E. Raw and F.W.G. Hill (Eds.), The Veterinary Annual, Vol. 28, Scientichnica, Toronto, pp. 43-48.

Flower, J.J., Moncada, S. and Vane, J.R. 1980. Analgesic-antipyretics and anti-inflammatory agents. In: A. Goodman and L.S. Gillman (Eds.), Goodman & Gilman's the pharmacological basis of therapeutics, MacMillan Publishing, New York, pp. 689.

Freysz, M., Temour, Q., Mazze, R.I., Bertrix, L., Cohen, S., Samii, K. and Faucon, G. 1989. Potentiation by mild hypothermia of ventricular conduction disturbances and reentrant arrhythmias induced by bupivacaine in dogs, Anesthesiology, 70 799-804.

Frohm, K.D. and Wallnau, L.B.x. 1983. Opiate effects on isolation induced hyperthermia, Pharm Biochem and Behav, 19 163-167.

Gallup, G.G. and Suarez, S.D. 1980. An ethological analysis of open-field behaviour in chickens, An Behav, 28 368-378.

Garrettson, P.R. and Rohwer, F.C. 1998. Reproductive effort and survival of wild blue-winged teal *Anas discors*, with backpack harness and implant transmitters, Can Field Nat, 112 212-216.

Gartner, K., Buttner, D., Dohler, K., Friedel, R., Lindena, J. and Trautschold, I. 1980. Stress response of rats to handling and experimental procedures, Lab An, 14 267-274.

Gentle, M. 1991. The acute effects of amputation on peripheral trigeminal afferents in Gallus gallus var domesticus, Pain, 46 97-103.

Gentle, M.J. 1989. Cutaneous sensory afferents recorded from the nervus intramandibularis of Gallus gallus var domesticus, Comp Physiol A, 164 763-774.

Gentle, M.J. 1991. Behavioural and physiological responses to pain in the chicken, Acta XX Congresssus Internationalis Ornithologici.

Gentle, M.J. 1992. Pain in birds, Animal Welfare, 1 235-247.

Gentle, M.J. 1997. Pain-related behaviour following sodium urate arthritis is expressed in decerebrate chickens, Physiol Behav, 62 581-584.

Gentle, M.J. 1997. Sodium urate arthritis: effects on the sensory properties of articular afferents in the chicken, Pain, 70 245-251.

Gentle, M.J. and Corr, S.A. 1995. Endogenous analgesia in the chicken, Neurosci Lett, 201 211-214.

Gentle, M.J., David, W., Hunter, L.N. and Jones, R.B. 1990. Behavioural evidence for persistent pain following partial beak amputation in chickens, Appl An Behav Sci, 27 149-157.

Gentle, M.J. and Hill, F.L. 1987. Oral lesions in the chicken: behavioural responses following nociceptive stimulation, Physiol Behav, 40 781-783.

Gentle, M.J., Hughers, B.O. and Hubrecht, R.C. 1982. The effect of beak-trimming on food intake, feeding behaviour and body weight in adult hens, Appl An Ethol, 8 147-159.

Gentle, M.J. and Hunter, L.N. 1990. Physiological and behavioural responses associated with feather removal in Gallus gallus var domesticus, Res Vet Sci, 50 95-101.

Gentle, M.J. and Hunter, L.N. 1993. Neurogenic inflammation in the chicken (Gallus gallus var domesticus), Comp Biochem Physiol C, 105 459-62.

Gentle, M.J., Jones, R.B. and C., W.S. 1989. Physiological changes during tonic immobility in Gallus gallus var domesticus, Physiol Behav, 46 843-847.

Gentle, M.J. and Thorp, B.H. 1994. Sensory properties of ankle joint capsule mechanoreceptors in acute monoarthritic chickens, Pain, 57 361-374.

Gentle, M.J. and Tilston, V.L. 1999. Reduction in peripheral inflammation by changes in attention, Physiol Behav, 66 289-292.

Glasser, R.S., Knego, R.S., Delashaw, J.B. and Fessler, R.G. 1993. The perioperative use of corticosteroids and bupivacaine in the management of lumbar disc disease, J Neurosurg, 78 383-387.

Glatz, P.C. 1987. Effects of beak trimming and restrain on heart rate, food intake, body weight and egg production in hens, Br Poult Sci, 28 601-611.

Glatz, P.C., Murphy, B.L. and Preston, A.P. 1992. Analgesia therapy of beak-trimmed chickens, Aust Vet J, 69 18.

Gloutney, M.L. 1996. Nest-site selection, energetics of reproduction and cmparative behaviour of mallard (Anas platyrhynchos) and blue-winged teal (A. discors), Dept of Biology, University of Saskatchewan, Saskatoon, pp. 225.

Gloutney, M.L., Clark, R.G., Afton, A.D. and Huff, G.J. 1993. Timing of nest serches for upland nesting waterfowl, J Wildl Manage, 57 597-601.

Goodman, N.W., Black, A.S.N. and Carter, J.A. 1987. Some ventilatory effects of propofol in the anaesthetised dog, Br J Anaesth, 63 87-92.

Gottschaldt, K.-M., Fruhstorfer, H., Schmidt, W. and Kraft, I. 1982. Thermosensitivity and its possible fine-structural basis in mechanoreceptors in the beak skin of geese, J Comp Neurol, 205 219-245.

Govrin-Lippmann, R. and Devor, M. 1978. Ongoing activity in severed nerves: source and variation with time, Brain Res, 159 406-410.

Gratto-Trevor, C.L., Oring, L.W. and Fivizzani, A.J. 1991. Effects of blood sampling on hormone levels in the semipalmated sandpiper, J Field Ornithol, 62 19-27.

Greenwood, R.J. 1974. Reproductive aspects, growth and developement of Greenland mallards, Condor, 76 223-225.

Greenwood, R.J. and Sargeant, A.B. 1973. Influence of radio packs on captive mallard and blue-winged teal, J Wildl Manage, 37 3-9.

Gregory, J.E. 1973. An electrophysiological investigation of the receptor apparatus in the ducks bill, J Physiol, 229 151-164.

Grisneaux, E., Pibarot, P., Dupuis, J. and Blais, D. 1999. Comparison of ketoprofen and carprofen administered prior to orthopedic surgery for control of postoperative pain in dogs, J Am Vet Med Assoc, 215 1105-1110.

Groppetti, A., Braga, P.C. and Biella, G. 1988. Effect of aspririn on serotonin and met-enkephalin in brain: correlation with the antinociceptive activity of the drug, Neuropharm, 27 499-505.

Gupta, R.N. and Dauphin, A. 1994. Column liquid chromatographic determination of bupivacaine in human serum using solid-phase extraction, J Chromatography B: Biomed Appl, 658 113-119.

Haftorn, S. and Reinertsen, R.E. 1982. Regulation of body temperatrue and heat transfer to eggs during incubation, Ornis Scand, 13 1-10.

Hall, M.R. 1987. External stimuli affecting incubation behavior and prolactin secretion in the duck (Anas platyrhynchos), Horm Behav, 21 269-287.

Haramis, G.M., Nichols, J.D., Pollock, K.H. and Hines, J.E. 1986. The relationship between body mass and survival of wintering canvasbacks, Auk, 103 506-514.

Hardee, M.M. and Moore, J.N. 1986. Effects of flunixin meglumine, phenylbutazone and a selective sunthetase inhibitor (UK-38,485) on thromboxane and prostacyclin production in healthy horses, Res Vet Sci, 40 152-156.

Hargreaves, R.C. and Champion, L.R. 1965. Debeaking of caged layers, Poult Sci, 44 1223-1227.

Harris, K. 1992. The role of prostaglandins in the control of renal functions, Br J Anaesth, 69 233-235.

Hay Kraus, B.L., Greenblatt, D.J., Venkatakrishnan, K. and Court, M.H. 2000. Evidence for propofol hydroxylation by cytochrome P4502B11 in canine liver microsomes: breed and gender differences, Xenobiotica, 30 575-588.

Hayes, R.L., Dubner, R. and Hoffman, D.S. 1981. Neuronal activity in the medullary dorsal horn of awake monkeys trained in a thermal discrimination task. II. Behavioural modulation of response to thermal and mechanical stimuli., J Neurophysiol., 52 170-187.

Hendrickson, C.M. and Lin, S. 1980. Opiate receptors in highly purified neuronal cell populations isolated in bulk form embryonic chick brain, Neuropharmacology, 13 731-739.

Herling, S., Coale, E.H., Valentino, R.J., Hein, D.W. and Woods, J.H. 1980. Narcotic discrimination in pigeons, J Pharmacol Exp Therap, 214 139-146.

Heusmann, H.W., Burrell, R.G. and Bellville, R. 1978. Automatic short-term color marking for nesting wood ducks, J Wildl Manage, 42 429-432.

Higgins, A.J. and Lees, P. 1984. The acute inflammatory process, arachidonic acid metabolism and th emode of action of anti-inflammatory drugs, Eq Vet J, 16 163-175.

Hocking, P.M., Bernard, R. and Maxwell, M.H. 1999. Assessment of pain during locomotion and the welfare of adult male turkeys with destructive cartilage loss of the hip joint, Br Poult Sci, 40 30-34.

Hocking, P.M., Gentle, M.J., Bernard, R. and Dunn, L.N. 1997. Evaluation of a protocol for determining the effectiveness of pretreatment with local analgesics for reducing experimentally induced articular pain in domestic fowl, Res Vet Sci, 63 263-267.

Hofer, H. and East, M.L. 1998. Biological conservation and stress. In: A.P. Moller, M. Milinski and P.J.B. Slater (Eds.), Advances in the study of behaviour: stress and behavior, Vol. 27, Academic Press, New York, pp. 405-525.

Holloway, J.A., Trought, C.O., Wright, L.E. and Keyser, G.F. 1980. Cutaneous receptive field characteristics of primary affterents and dorsal horn cells in the avian (Gallus domesticus), Exp Neurol, 68 477-488.

Holmes, W.N. and Phillips, J.G. 1976. The adrenal cortex of birds. In: C.I. Jones and I.W. Henderson (Eds.), General, Comparative and Clinical Endocrinology of the Adrenal Cortex, Vol. 1, Academic Press, New York, pp. 293-413.

Houston, R.A. and Greenwood, R.J. 1993. Effects of radio transmitters on nesting captive mallards, J Wildl Manage, 57 703-709.

Hughes, R.A. 1990. Codiene analgesic and morphine hyperalgesic effects on thermal nociception in domestic fowl, Pharm Biochem Behav, 35 567-570.

Hughes, R.A. 1990. Strain-dependent morphine-induced analgesic and hyperalgesic effects on thermal nociception in domestic fowl (Gallus gallus), Behav Neruosci, 104 619-624.

Hughes, R.A. 1990. Strain-dependent morphine-induced analgesic and hyperalgesic effects on thermal nociception in domestic fowl (Gallus gallus), Behav Neurosci, 104 619-624.

Hughes, R.A. and Baker, M.R. 1995. Baseline performance as a determinant of morphine hypo- and hyperalgesia in domestic fowl, Exp Clin Psychopharmacol, 3 3-8.

Hughes, R.A., Bowes, M. and Sufka, K.J. 1992. Morphine hyperalgesic effects on developmental changes in thermal nociception and respiration in domestic fowl (Gallus gallus), Pharmacol Biochem Behav, 42 535-539.

Hughes, R.A. and Sufka, K. 1990. The ontogeny of thermal nociception in domestic fowl: thermal stimulus intensity and isolation effects, Develop Psychobiol, 23 129-140.

Hughes, R.A. and Sufka, K.J. 1991. Morphine hyperalgesic effects on the formalin test in domestic fowl (Gallus gallus), Pharmacol Biochem Behav., 38 247-51.

Hutson, P.H., Curzon, G. and Ticklebank, M.D. 1984. Anti-nociception induced by brief foot-shock: characterization and roles of 5-hydroxytryptamine and dopamine. In: T.M. D. and G. Curzon (Eds.), Stress Induced Analgesia, John Wiley & Sons, Chichester, pp. 135-164.

Inoue, M., Satoh, W. and Murakami, H. 1997. Plasma alpha-1-acid glycoprotein in chickens infected with infectious bursal disease virus, Avian Dis, 41 164-170.

Jenkins, J.R. 1993. Post-operative care of the avian patient, Sem Avian Exotic Pet Med, 2 97-102.

Johnson, D.H., Nichols, J.D. and Schwartz, M.D. 1992. Population dynamics of breeding waterfowl. In: B.D.J. Batt, A.D. Afton, M.G. Anderson, C.D. Ankney, D.H. Johnson, J.A. Kadlec and G.L. Krapu (Eds.), Ecology and management of breeding waterfowl, University of Minnesota Press, Minneapolis.

Jones, M.W., Hodge, C.J.J., Apkarian, A.V. and Stevens, R.T. 1985. A dorsolateral spinothalamic pathway in cat, Brain Res, 335 188-193.

Jones, R.B. 1989. Chronic stressors, tonic immobility and leucocytic responses in the domestic fowl, Physiol Behav, 46 439-42.

Jones, R.B. and Harvey, S. 1987. Behavioural and adrenocortical responses of domestic chicks to systematic reduction in group size and to sequential disturbances of companions by experimenter, Behav Processes, 14 291-303.

Kanjhan, R. 1995. Opioids and pain, Clin Exp Pharm Physiol, 22 397-403.

Kantor, T.G. 1986. Ketoprofen: a review of its pharmacologic and clinical properties, Pharmacotherapy, 6 93-103.

Katz, J., Kavanagh, B.P., Sandler, A.N., Nierenberg, H., Boylan, J.F., Friedlander, M. and Shaw, B.F. 1992. Preemptive analgesia; clinical evidence of neuroplasticity contributing to post-operative pain, Anaesthesiology, 77 439-446.

Kauppila, T. 1998. Correlation between autotomy-behavior and current theories of neuropathic pain, Neurosci Biobehav Rev, 23 111-129.

Kear, J. 1965. Internal food reserves of hatching mallard ducklings, J Wildl Manage, 29 523-528.

Kehoe, P.A. and Blass, E.M. 1986. Opioid-mediation of separation distress in 10-day old rats: Reversal of stress with maternal stimuli, Develop Psychobiol, 19 385-398.

Kenward, R.E. 1987. Wildlife radio tagging., Academic Press, London, 222 pp.

Klein, P.N., Charmatz, K. and Langenberg, J. 1994. The effect of flunixin meglumine (Banamine) on the renal function in northern bobwhite (Colinus virginianus): an avian model, Proc Amer Assoc Zoo Vet, pp 128-131.

Kore, A.M. 1990. Toxicology of nonsteroidal anti-inflammatory drugs., Vet Clin North Am: Sm An Pract, 20 419-430.

Korschgen, C.E., Kenow, K.P., Green, W.L., Johnson, D.H., Samuel, M.D. and Sileo, L. 1996. Survival of radiomarked canvasback ducklings in northwestern Minnesota, J Wildl Manage, 60 120-132.

Korschgen, C.E., Maxson, S.J. and Kuechle, V.B. 1984. Evaluation of implanted transmitters in ducks, J Wildl Manage, 48 983-987.

Koskimies, J. and Lahti, L. 1964. Cold-hardiness of the newly hatched young in relation to ecology and distribution in ten species of European ducks, Auk, 81 281-307.

Krapu, G.L. 1981. The role of nutrient reserves in mallard reproduction, Auk, 98 29-38.

Krapu, G.L., Johnson, D.H. and Dane, C.W. 1979. Age determination of mallards, J Wildl Manage, 43 384-393.

Kumar, S. and Srivastava, S. 1965. Evaluation of hypoxia, hypercarbia and asphyxia in the production of cardiac arrest during surgical anesthsia, Ind J Physiol Pharm, 9 173-183.

Lamont, L.A., Tranquilli, W.J. and Grimm, K.A. 2000. Physiology of Pain, Vet Clin North Am: Sm An Pract, 30 703-728

Lamont, L.A., Tranquilli, W.J. and Mathews, K.A. 2000. Adunctive analgesic therapy, Vet Clin North Am: Sm An Pract, 30 805-813.

Lancaster, W.C. 1997. Systematics. In: R.B. Altman, S.L. Clubb, G.M. Dorrestein and K. Quesenberry (Eds.), Avian Medicie and Surgery, W. B. Saunders, Philadelphia.

Landoni, M.F., Comas, W., Mucci, N., Anglarilli, G., Bidal, D. and Lees, P. 1999. Enantiospecific pharmacokinetics and pharmacodynamics of ketoprofen in sheep, J Vet Pharmacol Therap, 22 349-359.

Landoni, M.F., Cunningham and Lees, P. 1995. Pharmacokinetics and pharmacodynamics of ketoprofen in calves applying PK/PD modelling, J Vet Pharmacol Therap, 18 315-324.

Landoni, M.F., Cunninghan, F.M. and Lees, P. 1995. Comparative pharmacodynamics of flunixin, ketoprofen and tolfenamic acid in calves, Vet Rec, 137 428-431.

Landoni, M.F., Foot, R., Frean, S. and Lees, P. 1996. Effects of flunixin, tolfenamic acid, R(-) and S(+) ketoprofen on the response of equine synoviocytes to lipopolysaccharide stimulation, Eq Vet J, 28 468-475.

Landoni, M.F. and Lees, P. 1995. Comparison of the anti-inflammatory actions of flunixin and ketoprofen in horses applying PK/PD modelling, Eq Vet J, 27 247-256.

Landoni, M.F. and Lees, P. 1995. Influence of formulation on the pharmacokinetics and bioavailability of racemic ketoprofen in horses, J Vet Pharmacol Therap, 18 446-450.

Landoni, M.F. and Lees, P. 1995. Pharmacokinetics and pharmacodynamics of ketoprofen enantiomers in calves, Chirality, 7 586-597.

Landoni, M.F. and Lees, P. 1996. Pharmacokinetics and pharmacodynamics of ketoprofen enantiomers in the horse, J Vet Pharmacol Therap, 19 466-474.

Le Maho, Y., Karmann, H., Briot, D., Hanrich, Y., Robin, J.-P., Mioskowski, E., cherel, Y. and Farni, J. 1992. Stress in birds due to routine handling and a technique to avoid it, Am J Physiol, 263 R775-R781.

Lees, P., Ewins, C.P., Taylor, B.O. and Sedgwick, A.D. 1987. Serum thromboxane in the horse and its inhibition by aspirin, phenylbutazone and flunixin, Br Vet J, 143 462-476.

Lees, P.'and Higgins, A.J. 1985. Clinical pharmacology and therapeutic uses of non-steroidal anti-inflammatory drugs in the horse, Eq Vet J, 17 83-96.

Lees, P. and Taylor, P.M. 1991. Pharmacodynamics and pharmacokinetics of flunixin in the cat, Br Vet J, 147 298-305.

Leitner, L.M. and Roumy, M. 1974. Thermosensitive units in the toungue and in the skin of the duck's bill, Pflugers Arch, 346 151-156.

Lemke, K.A. and Dawson, S.D. 2000. Local and Regional Anesthesia, Vet Clin North Am: Small An Pract, 30 839-858.

Leslie, K., Sessler, D.E., Bjorksen, A.R. and Moayeri, A. 1995. Mild hypothermia alters propofol pharmacokinetics and increases duration of atracurium, Anesth Analg, 80 1007-1014.

Lin, H.C., Benson, G.J., Thurmon, J.C., Trnaquilli, W.J., Olson, W.A. and Bevill, R.F. 1993. Influence of anesthetic regimens on the perioperative catecholamine response associated with onychectomy in cats, American Journal of Veterinary Research, 54 1721-1724.

Livingston, A. 1994. Physiological basis for pain perception in animals, J Vet Anaesth, 21 73-77.

Livingston, A. 2000. Mechanism of action of nonsteroidal anti-inflammatory drugs, Vet Clin North Am: Sm An Pract, 30 773-782.

Livingston, A., Low, J. and Morris, B. 1984. Effects of clonidine and xylazine on body temperature in the rat, Br J Pharmacol, 81 189 - 195.

Lofstrom, J. 1978. Tissue distribution of local anesthetics with special reference to the lung, Int Anesthesiol Clin, 16 53-71.

Lokemoen, J.T., Duebbert, H.F. and Sharp, D.E. 1990. Homing and reproductive habits of mallards, gadwalls and blue-winged teal, Wildl Monogr, 106 1-28.

Ludders, J.W. and Matthews, N. 1996. Birds. In: J.C. Thurmon, W.J. Tranquiili and G.J. Benson (Eds.), Lumb and Jones' Veterinary Anesthesia, Williams and Wilkins Co., Baltimore, pp. 183-209.

Ludders, J.W., Mitchell, G.S. and Rode, J. 1990. Minimal anesthetic concentration and cardiopulmonary dose response of isoflurane in ducks, Vet Surg, 19 304-307.

Ludders, J.W., Rode, J. and Mitchell, G.S. 1989. Isoflurane anesthesia in sandhill cranes (Grus canadensis): minimal anesthetic concentration and cardiopulmonary dose-response during spontaneous and controlled breathing, Anesth Analg, 68 511-516.

Luna, L. G. 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology, McGraw-Hill Book Company, New York.

Machin, K.L., Assessment of pain in captive and free-ranging ducks after intra-abdominal transmitter placement, Proc Am Assoc Zoo Vet and Am Assoc Wildl Vet, Joint Conference, 1998.

Machin, K.L. and Caulkett, N.A. 1998. Cardiopulmonary effects of propofol and a medetomidine-midazolam-ketamine combination in mallard ducks, Am J Vet Res, 59 598-602.

Machin, K.L. and Caulkett, N.A. 2000. Evaluation of isoflurane and propofol anesthesia for intraabdominal transmitter placement in nesting female canvasback ducks, J Wildl Dis, 36 324-334.

Machin, K.L., Tellier, L.A., Lair, S. and Livingston, A. 2001. Pharmocodynamics of flunixin and ketoprofen in mallard ducks, J Zoo Wildl Med, 32 222-229.

Mansour, A., Khachaturian, L.M.E., Akil, H. and Watshon, S.J. 1988. Anatomy of CNS opioid receptors, Trend Neurosci, 11 308-314.

Martin, R. 1984. Pharmacology of opioids, Pharmacol Rev, 35 283-323.

Martini, A., Bondiolotti, G.P. and Sacerdote, P. 1984. Diclofenac increases beta-endorphin plasma concentrations, J Int Med Res, 12 92-95.

Mather, L.E. and Cousins, M.J. 1979. Local anesthetics and their current clinical use, Drugs, 18 185 - 205.

Mathews, K.A. 2000. Nonsteroidal anti-inflammatory analgesics: indications and contraindications for pain management in dogs and cats, Vet Clin North Am: Small An Pract, 30 783-804.

Mauser, D.M. and Jarvis, R.L. 1991. Attaching radio transmitters to 1-day-old mallard ducklings, J Wildl Manage, 55 488-491.

Mawhinney, K. and Diamond, A.W. 1999. Using radio-transmitters to improve estimates of gull predation on common eider ducklings, Condor, 101 824-831.

Maze, M. and Tranquilli, W. 1991. Alph-2 adrenoceptor agonist: defining the role in clinical anesthesia, Anesthesiology, 74 581 - 605.

McGeown, D., Danbury, T.C., Waterman-Pearson, A.E. and Kestin, S.C. 1999. Effect of carprofen on lameness in broiler chickens, Vet Rec, 144 668-671.

McKellar, Q.A., Galbraith, E.A., Bogan, J.A., Russell, C.S., Hooke, R.E. and Lees, P. 1989. Flunixin pharmacokinetics and serum thromboxane inhibition in the dog, Vet Rec, 124 651-654.

Meade, R. 1996. Canvasback incubation constancy, factors affecting canvasback incubation constancy and canvasback egg hatchability, Louisiana State University, Baton Rouge, pp. 28.

Mendenhall, B.M. and Milne, H. 1985. Factors affecting ducking survival of eiders, Somateria mollissima in northeast Scotland, Ibis, 127 148-158.

Mickelson, P.G. 1975. Breeding biology of cacking geese and associated species on the Yukon-Kuskowin Delta, Alaska, Wildl Monogr, 45 1-35.

Milne, B. 1991. Alpha-2 agonists and anaesthesia, Can J Anaesth, 28 809 - 813.

Moseley, L.J. and Mueller, H.C. 1975. A device for color-marking nesting birds, Bird-banding, 46 341-342.

Muhammad, B.Y. and Kitchen, I. 1994. Effect of chronic maternal diazepam treatment on the development of stress-induced antinociception in young rats, Pharmacol Biochem Behav, 47 927-33.

Necker, R. and Reiner, B. 1980. Temperature-sensitive mechanoreceptors, thermoreceptors and heat nociceptors in the feathered skin of pigeons, J Comp Physiol, 135 201-207.

Neugebauer, B. and Schaible, H.G. 1988. Peripheral and spinal components of the sensitization of spinal neurons during an acute experimental arthritis, Agents. Actions, 25 234-236.

Nicol, G.D., Klingberg, D.K. and Vasko, M.R. 1992. Prostaglandin E2 increases in calcium conductance and stimulates release of substance P in avian sensory neurons, J Neurosci, 12 1917-1927.

Nolan, A.M. 2000. Pharmacology of analgesic drugs. In: P. Flecknell and A. Waterman-Pearson (Eds.), Pain Management in Animals, W. B. Saunders, New York, pp. 21-52.

Obrecht, H.H., Pennycuik, C.J. and Fuller, M.R. 1988. wind tunel experiments to assess the effect of back-mounted radio transmitters on bird body drag, J Exp Biol, 135 265-273.

Olsen, G.E., Dein, F.J., Haramis, G.M. and Jorde, D.G. 1992. Implanting radiotransmitters in wintering canvasbacks, J Wildl Manage, 56 325-328.

Ookawa, T. 1972. Polygraphic recordings during adult hen hypnosis, Poultry Sci, 51 853-958.

Orthmeyer, D.L. and Ball, I.J. 1990. Survival of mallard broods on benton lake national wildlife refuge in northcentral Montana, J Wildl Manage, 54 62-66.

Otsuka, M. and Yanagisawa, M. 1990. Pain and neurotransmitters, Cell Mol Neurobiol, 10 293-302.

Owens, J.G. and Kamerling, S.G. 1995. Effects of ketoprofen and phenylbutazone on chronic hoof pain and lameness in the horse, Eq Vet J, 27 296-300.

Owens, J.G., Kamerling, S.G. and Barker, S.A. 1995. Pharmacokinetics of ketoprofen in healthy horses and horses with acute synovitis, J Vet Pharm Therap, 18 187-195.

Panksepp, J., Heran, B., Conner, R., Bishop, P. and Scott, J.P. 1978. The biology of social attachments: Opiates alleviate separation distress, Biol Psychiatry, 13 607-618.

Paquette, G.A., Devries, J.H., Emery, R.B., Dowerter, D.W., Joynt, B.L. and Sankowski, T.P. 1997. Effects of transmitters on reproduction and survival of wild mallards, J Wildl Manage, 61 953-961.

Pascoe, P.J. 2000. Opioid Analgesics, Vet Clin North Am: Small An Pract, 30 757-772.

Pascoe, P.J. 2000. Perioperative pain management, Vet Clin North Am: Small An Pract, 30 917-949.

Pateromichelakis, S. and Rood, J.P. 1982. Prostaglandin E1-induced sensitization of A moderate pressure mechanoreceptors, Brain Res, 232 89-90.

Paul-Murphy, J. and Ludders, J.W. 2001. Avian Analgesia, Vet Clinics North Am: Exotic Pet Pract, 4 35 - 45.

Paul-Murphy, J.R., Brunson, D.B. and Miletic, V. 1999. Analgesic effects of butorphanol and buprenorphine in conscious African grey parrots (Psittacus erithacus erithacus and Psittacus erithacus timneh), American Journal of Veterinary Research, 60 1218-1221.

Paul-Murphy, J.R., Brunson, D.B. and Miletic, V. 1999. A technique for evaluating analgesia in conscious perching birds, Am J Vet Res, 60 1213-1217.

Payan, D.G. 1989. Neuropeptides and inflammation: the role of substance P, Ann Rev Med, 40 341-52.

Pehrsson, O. 1982. An experimental study of egg and duckling size, and subsequent survival and growth rate as a result of food quality of laying mallards, Int Congr Game Biol, 14 195-202.

Perry, M.C. 1981. Abnormal behavior of canvasbacks equipped with radio transmitters, J Wildl Manage, 45 786-789.

Peshin, P.K. and Hall, L.W. 1996. Short duration anaesthesia for minor procedures in dogs, J Vet Anesth, 23 70-74.

Petrusewicz, J., Turowski, M., Foks, H., Pilarski, B. and Kaliszan, R. 1995. Comparative studies of antiplatelet activity of nonsteroidal antiinflammatory drugs and new pyrazine CH- and NH-acids, Life Sci, 56 667-677.

Pieretti, S., Di Giannuario, A., Domenici, M.R., Sagratella, S., Capasso, A., Sorrentino, L. and Loizzo, A. 1994. Dexamethasone-induced selective inhibition of the central mu opioid receptor: functional in vivo and in vitro evidence in rodents, Br J Pharmacol, 113 1416-1422.

Pietz, P.J., Krapu, G.L., Greenwood, R.J. and Lokemoen, J.T. 1993. Effects of harness transmitters on behavior and reproduction of wild mallards, J Wildl Manage, 57 696-703.

Plumb, D.C. 1995. Veterinary Drug Handbook, Iowa State Press, Ames, 790 pp.

Prince, H.H. 1965. The breeding ecology of wood duck (Aiz sponsa L.) and common goldeneye (Bucephala clangula L.) in central New Brunsiwick, University of New Brunswick, Fredericton.

Putman, R.R. 1995. Ethical considerations and animal welfare in ecological field studies, Biodiversity Conserv, 4 903-915.

Pyorala, S., Laurila, T., Lehtonen, S., Leppa, S. and Kaartinen, L. 1999. Local tissue damage in cows after intramuscular administration of preparations containing phenylbutazone, flunixin, ketoprofen and metamizole, Acta Vet Scand, 40 145-150.

Quasha, A.L., Eger, E.I.I. and Tinker, J.H. 1980. Determination and applications of MAC, Anesthsiology, 53 315-334.

Ragsdale, D.S., McPhee, J.C., Scheuer, T. and Catterall, W.A. 1994. Molecular determinants of state-dependant block of Na+ channels by local anesthetics, Science, 265 1724 - 1728.

Rawlings, N.C., Jeffcoate, I.A. and Rieger, D.L. 1984. The influence of estradiol-17B and progesterone on peripheral serum concentrations of luteinizing hormone and follicle stimulating hormone in the ovariectomized ewe, Theriogenology, 22 473-488.

Redig, P. 1982. An overview of avian anesthesia, Proc Am Assoc Zoo Vet, pp. 162-164..

Reim, D.A. and Middleton, C.C. 1995. Use of butorphanol as an anesthetic adjunc in turkeys, Lab An Sci, 45 696-697.

Reiner, A., Davis, B.M., Brecha, N. and Karten, H. 1984. The distribution of enkephalinlike immunoreactivity in the telencephalon of the adult and developing domestic chicken, J Comp Neuro, 228 245-262.

Rhymer, J.M. 1988. The effect of egg size variability on thermoregulation of mallard (*Anas platyrhynchos*) offspring and its implications for survival, Oecologia, 75 20-24.

Richner, H., Schneiter, P. and Stirnimann, H. 1989. Life-history consequences of growth rate depression: an experimental study on carrion crows (Corvus corone corone L.), Funct Ecol, 3 617-624.

Ringelman, J.K. and Flake, L.D. 1980. Diurnal visibility and activity of blue-winged teal and mallard broods, J Wildl Manage, 44 822-829.

Ritchie, B.W. and Harrison, G.J. 1994. Formulary. In: B.W. Ritchie, G.J. Harrison and L.R. Harrison (Eds.), Avian Medicine: principles and application, Wingers Publishing, Lake Worth, Florida, pp. 457-478.

Robertson, K.E., Cros, P.J. and Terry, J.C. 1985. The crucial first days, Am J Nursing, 85 30-47.

Rosland, J.H., Hunskaar, S. and Hole, K. 1987. The effect of diazepam on nociception in mice, Pharmacol Toxicol, 61 111-115.

Rotella, J.J., Howerter, D.W., Sankowski, T.P. and Devries, J.H. 1993. Nesting effort by wild mallards with 3 types of radio transmitters, J Wildl Manage, 57 690-695.

Rotella, J.J. and Ratti, J.T. 1990. Use of methoxyflurane to reduce nest abandonment of mallards, J Wildl Manage, 54 627-628.

Rotella, J.J. and Ratti, J.T. 1992. Mallard brood movements and wetland selection in southwestern Manitoba, J Wildl Manage, 56 508-515.

Rotella, J.J. and Ratti, J.T. 1992. Mallard brood survival and wetland habitat conditions in southwestern Manitoba, J Wildl Manage, 56 499-507.

Samour, J.H., Jones, D.M., Knight, J.A. and Howlett, J.C. 1984. Comparative studies of the use of some injectable anesthetic agents in birds, Vet Rec, 115 6-11.

Samuel, M.D., Goldberg, D.R., Thomas, C.B. and Sharp, P. 1995. Effects of Mycoplasma anatis and cold stress on hatching success and growth of mallard ducklings, J Wildl Dis, 31 172-178.

SAS, I.I. 1990. SAS user's guide: statistics. Version 6, SAS Institute Inc, Cary.

Scadding, J.W. 1981. Development of ongoing activity, mechanosesitivity, and adrenaline sensitivity in severed peripheral nerve axons, Exp Neurol, 73 345-364.

Schneider, C. 1961. Effects of morphine-like drugs in chicks, Nature 607-608.

Scott, D.B., McClure, J.H., Giasi, R.M., Seo, J. and Covino, B.G. 1980. Effects of concentration of local anaesthetic drugs in extradural block, Br J Anaesth, 52 1033-1037.

Scott, M.L. 1973. Nutrition in reproduction - direct effects and predictive functions. In: D.S. Farner (Ed.), Breeding biology of birds, National Academy of Science, Washington, pp. 46-49.

Sebel, P.S. and Lowdon, J.D. 1989. Propofol: a new intravenous anesthetic, Anesth, 71 260-277.

Sedinger, J.S. 1992. Ecology of prefledging waterfowl. In: B.D.J. Batt, A.D. Afton, M.G. Anderson, C.D. Ankney, D.H. Johnson, J.A. Kadlec and G.L. Krapu (Eds.), Ecology and management of breeding waterfowl, University of Minnesota Press, Minneapolis, pp. 109-127.

Seltzer, Z., Paran, Y., Elson, A. and Ginzburg, R. 1991. Neuropathic pain behaviour in rats depends on the afferent input from nerve-end neuroma including histamine-sensitive C-fibres, Neurosci Lett, 128 203-206.

Semrad, S.D., Hardee, G.E., Hardee, M.S. and Moore, J.N. 1985. Flunixin meglumine given in small doses: pharmacokinetics and prostaglandin inhibition in healthy horses, Am J Vet Res, 46 2474-2479.

Seymour, N.R. 1982. Mortality of duckling attributed to separation from mother and subsequent protracted exposure to low ambient temperature., Auk, 99 383-384.

Sharp, D.E. and Lokemoen, J.T. 1987. a decoy trap for breeding-season mallards in North Dakota, J Wildl Manage, 51 711-715.

Siddell, P.J. and Cousins, M.J. 1995. Recent advances in pain management, Aust New Zeal J Surg, 65 674-685.

Skarda, R.T. 1996. Local and regional anesthetic and analgesic techniques: Dogs. In: J.C. Thurmon, W.J. Tranquilli and G.J. Benson (Eds.), Lumb and Jones' Veterinary Anesthesia, Williams and Wilkins, Baltimore, pp. 426-447.

Slade, J.M. 1994. Epidural bupivacaine for aortic surgery, the effect of dilution on the quality of analgesia, Anaesthesia, 49 21-24.

Smith, J.D., Allen, S.W. and Quandt, J.E. 1999. Changes in cortisol concentration in response to stres and postoperative pain in client-owned cats and correlation with objective clinical variables, Am J Vet Res, 60 432-436.

Smullin, D.H., Skilling, S.R. and Larson, A.A. 1990. Interactions between substance P, calcitonin gene-related peptide, taurine and excitatory amino acids in the spinal cord, Pain, 42 93-101.

Soma, L.R., Uboh, C.E., Rudy, J. and Fegely, J. 1992. Plasma concentrations of flunixin in the horse: its relationship to thromboxane B2 production, Journal of Veterinary Pharmacology and Therapy, 15 292-300.

Soraci, C.E., Fogel, F. and Tapia, O. 2000. Chiral inversion of R(-) fenoprofen and ketoprofen enantiomers in cats, J Vet Pharmacol Therap, 23 265-271.

Sorenson, L.G., Nolan, P.M., M., B.A., Derrickson, S.R. and L., M.S. 1997. Hormonal dynamics during mate choice in the northern pintail: a test of the 'challenge' hypothesis, An Behav, 54 1117-1133.

Sosnowski, M., Lebrun, P. and Fodderie, L. 1992. Receptors, neuropathways, and mechanisms, Anesth. Clin. North. Am, 10 211-227.

Stephenson, R. 1994. Diving energetics in lesser scaup (*Aythya affinis*), J Exp Biol, 190 155-178.

Swan, G.E, Short, C.R. and Tubbesing, U.H. 1995. Non-steroidal anti-inflammatory drugs in domestic animals: disposition and clinical indications, J South African Vet Assoc, 66 35-41.

Stewart, P.A. and Wiley, M.J. 1981. Structural and histochemical features of the avian blood-brain barrier, J Comp Neurol, 202 157-167.

Stoskopf, M.K. 1994. Pain and analgesia in birds, reptiles, amphibians, and fish, Invest Ophthatlmol Vis Sci, 35 775-780.

Suesa, N., Fernandez, M.F., Gutierrez, M., Rufat, M.J., Rotllan, E., Calvo, L., Mauleon, D. and Carganico, G. 1993. Stereoselective cyclooxygenase inhibition in cellular models by the enantiomers of ketoprofen, Chirality, 5 589-595.

Sufka, K.J. and Hughes, R.A. 1990. Dose and temporal parameters of morphine-induced hyperalgesia in domestic fowl, Physiol Behav, 47 385-387.

Sufka, K.J. and Hughes, R.A. 1991. Differential effects of handling on isolation-induced vocalizations, hypoalgesia, and hyperthermia in domestic fowl, Physiol Behav, 50 129-133.

Sufka, K.J., Hughes, R.A. and Giordano, J. 1991. Effects of selective opiate antagoinists on morphine-induced hyperalgesia in domestic fowl, Pharmacol Biochem Behav, 38 49-54.

Sufka, K.J. and Weed, N.C. 1994. Construct validation of behavioral indices of insolation stress and inflammatory nociception in young domestic fowl, Physiol Behav, 55 741-746.

Svendsen, O., Rasmussen, F., Nielsen, P. and Steiness, E. 1979. The loss of creatine phosphokinase (CK) from intramuscular injection sites in rabbits. A predictive tool for local toxicity, Acta Pharmacol Toxicol Copenhagen, 44 324-328.

Swan, G.E., Short, C.R. and Tubbesing, U.H. 1995. Non-steroidal anti-inflammatory drugs in domestic animals: II disposition and clinical indications, J S Afr Vet Assoc, 66 35-41.

Szolcsanyi, J., Sann, H. and Pierau, F.-K. 1986. Nociception in pigeons is not impaired by capsaicin, Pain, 27 247-260.

Takahashi, K., Akiba, Y. and Tamura, K. 1997. Effect of dietary ascorbic acid on the hepatic microsomal mixed function oxidase system in liver of chicks treated with Escherichia coli lipopolysaccharide, Comp Biochem Physiol C: Pharmacol Toxicol Endocrin, 118 301-304.

Takahashi, K., Kaji, N., Akiba, Y. and Tamura, K. 1994. Plasma alpha 1-acid glycoprotien concentration in broilers: influence of age, sex and injection of Escheriachia coli lipopolysaccharide, Br Poult Sci, 35 427-432.

Talent, L.G., Jarvis, R.L. and Krapu, G.L. 1983. Survival of mallard broods in south-central North Dakota, Condor, 85 74-78.

Taylor, M.B., Ground, R.M., Mulrooney, P.D. and Morgan, M. 1986. Ventilatory effects of propofol druing induction of anaesthesia: comparison with thiopentone, Anaesthesia, 41 816-820.

Taylor, P.M., Winnard, J.G., Jeffries, R. and Lees, P. 1994. Flunixin in the cat: a pharmacodynamic, pharmacokinetic and toxicological study, Br Vet J, 150 253-262.

Thrumon, J.C., Ko, J.C.H., Benson, G.J., Tranquilli, W.J. and Olson, W.A. 1994. Hemodynamic and analgesic effects of propofol infusion in medetomidine-premedicated dogs, Am J Vet Res, 55 363-367.

Tobler, I. and Borbely, B.B. 1988. Sleep and EEG spectra in pigeon (Columba livia) underbadeline conditions and after sleep deprivation, J Comp Physio A, 163 729-738.

Tomchek, L.A., Hartman, D.A., Lewin, A.C., Calhoun, W., Chau, T.T. and :, C.R.P. 1991. Role of corticosterone in modulation of eicosanoid biosynthesis and antiinflammatory activity by 5-lipoxygenase (5-LO) and cyclooxygenase (CO) inhibitors, Agents Actions, 34. 155-159.

Torebjork, H.E., LaMotte, R.H. and J., R.C. 1984. Peripheral neural correlated of magnitude of cutaneious and hyperalgesia: simultaneous recordings in humans of pain and evoked responses in nociceptors with C-fibres, J Neurophysiol, 51 325-339.

Toutain, P.L., Autefage, A., Legrand, C. and Alvinerie, M. 1994. Plasma concentrations and therapeutic efficacy of phenylbutazone and flunixin meglumine in the horse: pharmacokinetic/pharmacodynamic modelling., J Vet Pharmacol Therap, 17 459-469.

Untergasser, G. and Hayward, J.S. 1972. Development of thermoregulation in ducklings, Can J Zool, 50 1243-1250.

Van Luitelaar, E.L.J.M., Vand Der Grinten, C.P.M., Blokhuis, H.J. and Coenen, A.M.L. 1987. Sleep in the domestic hen (Gallus domesticus), Physiol Behav, 41 409-414.

Vane, J.R. 1971. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs, Nature, 231 232-235.

Vasseur, P.B., levy, G., Dowd, E. and Eliot, J. 1988. Surgical wound infection rates in dogs and cats from a teaching hospital, Vet Surg, 17 60-64.

Virtanen, R. 1989. Pharmacological profiles of medetomidine and its antagonist, atipamezole, Acta Vet Scand, 85 29 - 37.

Vuyk, J., Oostwouder, C.J., Vletter, A.A., Burm, A.G.L. and Bovill, J.G. 2001. Gender differences in the pharmacokinetics of propofol in elderly patients during and after continuous infusion, Br J Anaesth, 86 183-188.

Walker, C.H.. 1998. Avian forms of cytochrome P450, Comp Biochem Physiol C: Pharmacol Toxicol Endocrin, 120 374-377.

Wall, P.D. and Gutnick, M. 1974. Ongoing activity in peripheral nerves: the physiology and pharmacology of impulses originating from a neuroma, Exp Neurol, 43 580-593.

Wan, Q. and Pang, S.F. 1994. Segmental, coronal and subcellular distribution of 2-[125I]iodomelatonin binding sites in the chicken spinal cord, Neurosci Lett, 180 253-256.

Watson, A., Nicholson, A., Church, D. and Pearson, M. 1996. Use of anti-inflammatory and analgesic drugs in dogs and cats, Aust Vet J, 74 203-210.

Watson, G.S. and Sufka, K.J. 1996. Chlordiazepoxide reverses social-separation-induced distress vocalizations and analgesia in young domestic fowl, Exp Clin Psychopharmacol, 4 347-353.

Weller, M.W. 1956. A simple field candler for waterfowl eggs, J Wildl Manage, 20 111-113.

Weller, M.W. 1957. An automatic nest-trap for waterfowl, J Wildl Manage, 21 456-458.

Westerhof, I., Mol, J.A., Van den Brom, W.E., Lumeij, J.T. and Rijnberk, A. 1994. Diurnal rhythms of plasma corticosterone concentrations in racing pigeons (Columba livia domestica) exposed to different light regimes, and the influence of frequent blood sampling, Avian Dis, 38 428-434.

White, F.L. and Holmes, D.D. 1976. A comparison of ketamine and ketamine xylazine combination for effective surgical anesthesia in the rabbit, Lab An Sci, 26 804 - 806.

White, F.N. and Kinney, J.L. 1974. Avian incubation, Science, 186 107-115.

Willis, W.D., Kenshalo, D.R.J. and Leonard, R.B. 1979. The cells of origin of the primate spinothalamic tract, J Comp Neurol, 188 543-574.

Willis, W.D.J. 1988. Dorsal horn neurophysiology of pain, Ann NY Acad Sci, 531 76-89.

Wilmore, D.W., Long, J.M. and Mason, A.D. 1989. Stress in surgical patients as a neurophysiologic reflex response, Surg Gynecol Obstet, 142 257-269.

Woolf, C.J. 1987. Physiological, inflammatory and neuropathic pain, Adv Tech Standards Neurosurg, 15 39-62.

Woolf, C.J. 1989. Recent advances in pathophysiology of acute pain, Br J Anaesth, 63 139-146.

Woolf, C.J. and Chong, M.-S. 1993. Preemptive analgesia: treating postoperative pain by preventing the establishment of central sensitization, Anesth Analg, 77 362-379.

Woolf, C.J. and Thompson, S.W.N. 1991. The induction and maintenance of central sensitization is dependent on N-methyl-D-aspartic acid receptor activation: implications for the treatment of post-injury pain hypersensitivity states, Pain, 44 293-299.

Woolley, S.C. and Gentle, M.J. 1987. Physiological and behavioural responses in the hen (*Gallus domesticus*) to nociceptive stimulation, Comparative Biochemistry and Physiology, 88A 27-31.

Wright, E.M., Marcella, K.L. and Woodson, J.F. 1985. Animal pain and control, Lab An, May/June 20-36.

Wylie, L.M. and Gentle, M.J. 1998. Feeding-induced tonic pain suppression in the chicken: reversal by naloxone, Physiol Behav, 64 27-30.

Yerkes, T. 1996. The influence of female age, body mass, and ambient conditions on redhead incubation constancy, Condor, 100 62-68.

Zenitsky, G.D., 1993. Growth and behavior of redhead ducklings implanted with dummy radio transmitters, Iowa State University, Ames, pp. 145.

Zhai, S.-Y. and Atsumi, S. 1997. Large dorsal horn neurons which receive inputs from numerous substance P-like immunoreacitve axon terminals in the laminae I and II of the chicken spinal cord, Neuroscience Research, 28 147-154.

Zhang, Y., Eger II, E.I., Dutton, R.C. and Sonner, J.M. 2000. Inhaled anesthetics have hyperalgesic effects at 0.1 minimum alveolar anesthetic concentration, Anesthesia and Analgesia, 91 462-466.

11. APPENDIX: EFFECTS OF BACK-MOUNTED RADIO TRANSMITTERS WITH A SUBCUTANEOUS ANCHOR ON MALLARD DUCKLINGS

11.1 Abstract

Estimates of duckling survival rates are critical for effective waterfowl population management. Therefore, more accurate estimates of duckling survival may be obtained by radio-marking ducklings within the brood; however, transmitters may have deleterious effects. Most captive studies report few effects of transmitters but ducklings in these studies were raised in ideal conditions. Thus, I examined effects of radio transmitters on mallard ducklings (*Anas platyrhynchos*) raised in outdoor pens, exposed to natural weather conditions. Ducklings were hatched and raised by brood hens in outdoor pens on St. Denis National Wildlife Area, Saskatchewan. In 1997, 36 day-old ducklings (5 broods) were divided randomly into 2 matched groups, with half the ducklings in each brood receiving transmitters and half receiving no transmitter but handled for the same amount of time. In 1998, 154 day-old ducklings (21 broods) were divided randomly into 3 groups with one third of the brood receiving a transmitter, one third a sham surgery and one third a handled control. All mortality occurred within 9 days of hatching. In 1997, mortality in the transmitter (29%) and control (5%) group was affected only by adverse weather but power to detect a treatment effect was low. In

1998, 37% of transmitter ducklings died, versus 7% of sham and 4% of control ducklings. Duckling mortality in the transmitter group was significantly greater than in the control group but mortality did not differ between control and sham groups. Mortality was also greater during poor weather. Growth and body mass were significantly lower in ducklings with transmitters compared to controls suggesting sublethal effects of transmitters. Researchers should be very cautious when interpreting duckling survival rates from data derived from studies using radio transmitters on ducklings.

11.2 Introduction

Waterfowl broods are difficult to monitor because of high mobility (Dzus and Clark 1997; Rotella and Ratti 1992) and low visibility (Ringelman and Flake 1980). Females often are radio-marked and followed to give an estimate of duckling survival (Rotella and Ratti 1992). More accurate measurements of timing and causes of duckling mortality may be achieved by radio marking individual ducklings within the brood (Korschgen et al. 1996; Mawhinney and Diamond 1999) However, transmitters may have deleterious effects on wild ducklings making ducklings more susceptible to mortality from chilling (Bakken et al. 1996; Bakken et al. 1999), exhaustion (Talent et al. 1983), predation (Mawhinney and Diamond 1999; Talent et al. 1983) and disease (Mendenhall and Milne 1985). Captive studies report nil to little effect of transmitters but ducklings in these studies were maintained in ideal conditions (Davis et al. 1999;

Mauser and Jarvis 1991; Zenitsky 1993). Therefore, I examined effects of back-mounted radio transmitters on duckling survival when ducklings were exposed to natural weather conditions. Objectives also included an estimation of sublethal effects of transmitters on duckling growth by measuring several structural parameters and body mass.

11.3 Methods and Materials

All ducks were maintained in accordance with guidelines of the Canadian Council on Animal Care as defined by the Guide to the Care and Use of Experimental Animals, and experiments were approved by the University of Saskatchewan Animal Care Committee. Work was conducted from May to July 1997 and 1998 in outdoor pens on St. Denis National Wildlife Area, Saskatchewan (52° 20' N, 106° 10' W). Ducklings were hatched and brooded by adult female mallard (*Anas platyrhynchos*) ducks that were F_1 and F_2 progeny of wild adults. Ducks were housed in pens measuring 3 x 10 m with 30% water and 70 % dry land, the latter consisting primarily of brome grass. Shelter was provided by a plywood box and each pen was enclosed by approximately, a 45 cm high wall of plywood. The roof and walls above the plywood consisted of chicken wire allowing exposure to natural weather conditions.

In 1997, day-old ducklings (5 broods) were divided randomly into 2 matched groups where half the ducklings in each brood received back-mounted transmitters (1.45 - 1.75g) with a subcutaneous anchor (transmitter group), and the remainder received no transmitter but were handled for the same amount of time (control group). In 1998, 154

day-old ducklings (21 broods) were divided randomly into 3 groups with one third of the brood receiving a transmitter, one third a sham (including local anesthetic) and one third being a handled control. Ducklings in all groups were handled for an equal amount of time. In addition, ducklings were held in a cardboard box with a hot water bottle and towels to ensure that body temperature was maintained when the were away from the hen.

In all groups transmitters were placed within 12 hours of hatch. Anchors were placed subcutaneously between the scapulars as described previously by Mauser and Jarvis (Mauser and Jarvis 1991), after injection of bupivacaine (Marcaine, Sanofi Winthrop, Markham, Ontario Canada) local anesthetic (2mg/kg maximum dose, 0.5% solution diluted 50% with sterile saline) at the surgical site to control for operative and post-operative pain. The proportion of transmitter to duckling weight was kept relatively constant at roughly 5 % by attaching the smallest transmitters to the smallest ducklings and the larger transmitters to the largest ducklings. Surgery was performed under aseptic conditions. In the sham and surgery groups, the surgical area was isolated with the use of autoclave tape and prepared by removing one or two feathers and cleansing the exposed skin (< $5mm^2$) with 10 % povidone-iodine topical solution (Purdue Frederick Inc., Pickering, Ontario). In the sham group, an injection of bupivacaine local anesthetic was made into the region between the scapulars but no incision was made. Ducklings in all groups had web tags placed after topical administration of 2 % lidocaine jelly (Xylocaine jelly, AstraZenica, Mississauga, Ont, Canada) for individual identification.

Ducklings were weighed and measured prior to transmitter attachment. Ducklings were weighed using a triple beam balance scale daily for the first week and every 2 days thereafter for 27 days and an additional weight was taken on day 30. In addition, measurements to the nearest 0.1 mm of beak width, culmen, head length, and tarsus length were done every 2 days for the first 7 days, and every 4 days until day 27 and an additional measurement was taken on day 30.

The order of visits to pens and capture of ducklings was randomized daily. Disturbance was minimized as much as possible and measurements were not taken on days when it was raining as mortality is known to increase when ducklings are exposed to severe weather (Prince 1965; Seymour 1982).

Females were fed a commercial duck and goose grower diet (Federated Co-operatives Limited, Saskatoon, SK, Canada) in a tray elevated approximately 15 cm from the ground. Food resources may not be critical to ducklings immediately after hatch because they can use lipids in residual yolk and body tissues through 4 days of age (Sedinger 1992). To mimic natural conditions, pelleted duck and goose starter (Federated Co-operatives Limited, Saskatoon, SK, Canada) was restricted to 75% of energy requirements for the first 2 days and then provided with 110% for the remainder of the experiment. Chicken wire with 4.9 cm holes was placed in a dome over the tray for the first 2 days so that the ducklings could have access to the tray but the female was restricted access; chicken wire was removed on the third day. Weekly energy requirements for duckling growth were calculated following (Scott 1973). All ducks and ducklings also had free access to oyster shell grit. Access to a grassy area and an

artificial pond was not limited and ducklings were exposed to normal daily weather conditions. Water levels were maintained by pumping water from a nearby pond at least twice weekly

In 1997, ducklings were observed for 30 minutes daily for the first week and opportunistically thereafter. However, in 1998, the large number of ducklings and broods prevented regular observations, so observations were made opportunistically. Behaviour of ducklings in each treatment group was recorded every minute as locomotion (walking or swimming), foraging, comfort behaviour (preening, stretching), resting (sleeping, sitting or loafing) or out of sight (not visible to the observer). The behaviour of the majority of ducklings for either marked or unmarked groups was considered the behaviour of that group. It was impossible to distinguish sham from control ducklings in the unmarked group, therefore behaviour for these groups were pooled in the unmarked group. Each behaviour was calculated as percent time spent performing the above behaviours as matched groups (marked and unmarked) within each brood. In addition to the above classifications, every preening event was recorded and each event had to be separated by a different behaviour (i.e., walking, resting, etc.) to be recorded a preening event. Preening was calculated as preening event per ducking.

11.3.1 Statistical Analysis

Data for each year were analysed separately for because treatments differed between years. SAS (SAS 1990) was used for data analyses and a difference was

considered significant at $P \leq 0.05$ (actual P values are reported). Survival data was analysed using a G-test or Fisher's exact test. However, to examine duckling survival more closely, logistic regression (Proc CATMOD) was performed with treatment, sex, brood size, hatch date and indices of weather and ducking condition as independent variables. Indices of weather conditions and duckling body size were derived using principal components analyses (PCA). To create an index of weather condition for each year and for each duckling, the mean value of each of three weather variables, mean daily wind speed, cumulative daily precipitation, and minimum daily temperature, for the life of each duckling was used in the PCA. For each year, the first principal component (PC1) that described a range from cold, rainy, windy conditions to warm, dry, calm conditions was used as the weather condition index. For 1997 and 1998, PC1 explained 57% and 67% of the variation

An index of duckling body size was generated for both years using beak width and tarsus and head length in a PCA. In 1998, an index of duckling condition was obtained by using residuals derived by regressing initial duckling mass on PC1 which explained 78% of the variation in duckling size and all variables, beak width (0.58), tarsus (0.58) and head (0.59) length, loading positively. However, in 1997, initial body mass was used as an index of duckling condition as the regression of initial duckling mass on PC1, which explained 54% of the variation in duckling body size, was nonsignificant.

For both years, data (mass, beak width, culmen, and head and tarsus length) were $\log_{10}$ transformed to obtain slope by linear regression. Ducklings that lived for ≤ 3 days were excluded from the analysis. Only ducklings that lived to 30 days were included in

the 30 day analysis. Nested ANOVAs were performed to assess effects of treatment and sex on duckling growth as estimated by mass, beak width, culmen, and head and tarsus length.

The effect of transmitters on aspects of duckling behaviour were analysed using a multivariate analysis of variance (MANOVA) to control simultaneously for several dependent behaviour variables. If the MANOVA was significant, a univariate ANOVA was used to identify which aspects of behaviour varied between treatments.

11.4 Results

11.4.1 Survival

In both years, ducklings that died were found on dry land. In 1997, mortality in the transmitter group was significantly greater than the control group (Fisher's exact test, $P = 0.049$; Table 1). Of the 5 ducklings in the transmitter group that died, 3 were females (27 % of females) and 2 were male (40 % of males). One female duckling (8 % of females in the control group) in the control group also died. The mean time to death in the transmitter group was 3.0 ± 0.6 days (mean ± standard error) and no mortality occurred after day 5 (Figure 1). The duckling in the control group was found dead on the third day.

In 1998, there was a significant difference in mortality among groups (G-test = 24.03, df = 2, $P = 0.001$; Table 1). Of the 19 ducklings that died and had transmitters, 11 were female (46 % of females) and 10 were male (37 % of males). In the sham group, 1 was female (3 % of females) and 3 were male (13 % of males), and 2 were female (8 %

of females) in the control group. No mortality occurred after day 9 (Figure 1). The mean time to death in the transmitter group was 3.9 ± 2.7 days compared to 6.0 ± 2.7 days and 3.0 ± 1.0 days in the sham and control groups, respectively.

In 1997, duckling survival was negatively related to weather conditions (-1.71 ± 0.6; $\chi^2 = 7.77$, $P = 0.005$), with more ducklings dying during poor weather conditions. Treatment, brood size, duckling body mass and hatch date were not related to duckling survival. In 1998, no difference in survival between sham and control groups (0.65 ± 1.1, $\chi^2 = 0.32$, $P = 0.57$),was found thus these groups were pooled. Duckling mortality of ducklings with transmitters was greater compared with ducklings without transmitters (1.59 ± 0.4, $\chi^2 = 13.04$, $P = 0.0003$). Duckling survival was negatively related to weather conditions (-2.14 ± 0.5, $\chi^2 = 17.89$, $P < 0.0001$), with mortality corresponding to cold, wet and windy conditions. No treatment by weather interaction was detected. Duckling hatch date, body condition and brood size were not related to duckling survival.

11.4.2 Growth

In 1997, no difference in female hatch weight between treatments was found but male ducklings with transmitters were significantly heavier at hatch (Student's t test, P = 0.05) than males without transmitters. In 1998, no differences occurred in hatch weight among treatments, but females were weighed less than males (One-way ANOVA, df = 153,1; P = 0.026).

In 1997, ducklings without transmitters had a larger beak width slope compared to ducklings with transmitters (Partial F = 8.81; df = 1,29; P = 0.007) and head length was longer in males compared with females (Partial F = 4.73; df = 1, 26; P = 0.042). All other body size measures, including 30 day variables, did not differ between treatments or sex. In 1998, ducklings with transmitters had significantly smaller growth slopes of duckling mass (Partial F = 6.39; df = 2, 146; P = 0.0023; Fig 2.), tarsus length (partial F = 5.62; df = 2, 136; P = 0.0047), and beak width (partial F = 4.61; df = 2, 136; P = 0.012), compared with ducklings without transmitters (both sham and control groups). In addition, head length slope larger in males compared with females (F= 4.24; df = 1, 136; P = 0.42). Ducklings with transmitters were smaller at 30 days compared with ducklings without transmitters. Also males were larger than females but there was no treatment by sex interaction for duckling mass (treatment partial F = 16.66; df = 2, 123; P = 0.0001; sex partial F = 6.71; df = 1, 123; P = 0.01), head length (treatment partial F = 6.08, df = 1, 123; P = 0.003; sex partial F = 18.34, df = 1, 123; P = 0.0001), and beak width (treatment partial F = 2.1; df = 2, 123; P = 0.02; sex partial F = 18.34; df = 1, 123; P = 0.003; Table 2). Males were larger than females at 30-day measurements in tarsus length (partial F = 12.35, df = 1, 123; P = 0.0007) and beak length (partial F = 8.11, df = 1, 123; P = 0.005; Table 2).

11.4.3 Behaviour

In both years, ducklings with transmitters spent more time performing comfort behaviours compared to controls. In 1997, ducklings with transmitters preened the surgical area 8 times more often than ducklings without a transmitter (Wilcoxon test, 1-tailed, $p = 0.04$). Although not significant, ducklings with transmitters spent more time out of sight (12 %) and less time foraging (14 %) compared to ducklings in the control group (5 % and 17 %, respectively). In 1998, ducklings with transmitters preened the surgical area 5 times more often (Wilcoxon test, 1-tailed, $p < 0.001$). Ducklings with transmitters spent more time resting (33%) and less time foraging (16%) compared to ducklings in the control groups (27% and 22%, respectively; Table 3), but this was not significant.

In both years, ducklings with transmitters were also observed shivering, and pulling on the transmitter body and antenna. The hen was observed stepping on the antenna of ducklings with transmitters, and prevented them from moving on several occasions. All ducklings with transmitters had an area of swelling and feather loss associated with the subcutaneous anchor. No plumage disruption was noted in either the control or sham groups. Swelling was most prominent during the first 7 days after transmitter attachment. In addition, ducklings with transmitters had an area of wet feathers surrounding the transmitter anchor at the site of attachment.

11.5 Discussion

Ducklings with transmitters had higher mortality, decreased growth, and displayed more comfort behaviour than ducklings without transmitters, results which contradict other captive studies (Davis et al. 1999; Mauser and Jarvis 1991; Zenitsky 1993). Predation is often identified as an important source of duckling mortality (Talent et al. 1983) but poor weather also reduces duckling survival (Korschgen et al. 1996; Mendenhall and Milne 1985). Mortality in early brood rearing, reported here, is similar to other studies (Korschgen et al. 1996). Poor weather conditions were the primary cause of duckling mortality in 1997 but power to detect differences between treatments was likely low due to small sample size. Mortality in the control and sham groups in 1998 can be attributed primarily to weather conditions since predation was not a factor. Thus, poor weather conditions would only account for roughly 4 to 7 % of the mortality in the transmitter group.

Ducklings of dabbling duck species appear to be significantly less cold-tolerant than those of diving species (Koskimies and Lahti 1964; Seymour 1982). Lower limit of temperature tolerance for ducklings appears to be in the range from 0 - 10C (Bakken et al. 1999; Koskimies and Lahti 1964; Untergasser and Hayward 1972). In one study (Bakken et al. 1996), eight of twenty-four mallard ducklings became lethargic, had decreased oxygen consumption, and in some cases died when exposed to low temperatures (5 and 10 C).

Available cover has a major effect on wind exposure. Wind speed within grass or sedge cover typically is low at ground level. Ducklings in the wild use dense vegetation as much as possible. In cover, a duckling will be exposed to wind < 1 m/sec as long as free-stream wind does not exceed 15-25 m/sec (Bakken et al. 1999; Lokemoen et al. 1990). As a result, it is unlikely that wind played a major role in mortality in this study as all pens were surrounded by a 45 cm high barrier. Dead ducklings in this study were found in the open, unprotected by dense vegetation, therefore hypothermia may have been a factor. Observed cases of mortality, attributed to hypothermia, have involved ducklings swimming in open water (Seymour 1982). In this study, all ducklings that died in this study were found on land.

Evidence of increased heat loss was seen in ducklings with transmitters because they were observed shivering and had plumage disruption over the subcutaneous anchor. Increased heat loss must be balanced by heat production (Bakken et al. 1999; Koskimies and Lahti 1964), thus increasing energy requirements to maintain body temperature, which would decrease nutrients allocated to growth and weight gain. Mild cold stress can lead to reduced duckling growth even when given *ad libitum* food and water (Samuel et al. 1995). Mallard ducklings are not adequately adapted to survive in cool environments because of small body size, limited thermogenic capacity, and ineffective insulation compared to fully grown ducks (Koskimies and Lahti 1964). Ducklings with external transmitters show areas of increased surface temperature around the externally anchored transmitters in thermographic images, suggesting that heat loss may be increased in ducklings with transmitters (Bakken et al. 1996). Wet plumage, disrupted

feather growth and vascularization around the anchor may also have contributed to heat loss (Perry 1981; Zenitsky 1993), thereby increasing thermoregulatory costs in cold, wet, windy weather or when ducklings were not protected from cool conditions by the hen.

With the transmitter in place, the anchor prong can act as a wick, providing an avenue for infection. Ducklings with transmitters had some evidence of swelling or abscess associated with the anchor prong. Behaviour and thermoregulation could be affected by general malaise due to infections, which do not result in obvious external evidence of inflammation or disease (Bakken et al. 1996). Poor weather could also increase susceptibility to disease (Mendenhall and Milne 1985); and alter time-activity budgets (eg. increased time spent being brooded versus foraging), resulting in reduced foraging effort (Johnson et al. 1992).

Other contributing factors for duckling mortality and reduced growth in the transmitter group may have been increased energetic costs associated with wound healing (Bennett 1993), and/or transmitter weight (Obrecht et al. 1988). Physical exertion or movement resistance caused by transmitter size may also contribute to increased energy requirements (Obrecht et al. 1988). Transmitters in excess of 2.3 g visibly impaired mobility of newly hatched ducklings (Mauser and Jarvis 1991). In this study, increased energetic costs were reflected by the decreased growth of the surviving ducklings in the transmitter group. Differences between sexes in 30-day values were not unexpected since males are larger than females (Greenwood 1974).

Variation in survival probability is partly a function of body mass (Haramis et al. 1986). Large body mass is associated with good physical condition and a positive

energy budget (Haramis et al. 1986), the ability to withstand starvation and chilling (Pehrsson 1982; Rhymer 1988), protection against size-selective predation (Brown and Hunter 1985), and a dominant social status (Richner et al. 1989). Most mortality of ducklings occurs within the first 2 weeks of life (Orthmeyer and Ball 1990), which may, in part, be related to their small body size at that time (Brown and Hunter 1985). Any depression in growth rate, prolonging the length of time ducklings are in a vulnerable size class, may cause a proportionate increase in mortality (Brown and Hunter 1985; Zenitsky 1993).

In both years, the only significant behavioural difference was in the amount of time spent in comfort behaviour. Increased preening of the surgical site was evident in ducklings with transmitters. Increased time spent in comfort behaviour may have decreased time spent in other behaviours, including foraging which would have an impact on growth rates. Brooding behaviour was not often seen during observations. Young waterfowl may spend less time feeding during their first few days because they cannot thermoregulate effectively and require considerable brooding (Kear 1965), especially in adverse weather conditions. Observations were not performed in bad weather (temperatures <15 C, high wind speed, and during precipitation) because available physiological data and field studies suggest that exposure is a significant risk factor (Bakken et al. 1999). In addition, exposure of ducklings to severe weather is increased by brood separation due to disturbance (Prince 1965; Seymour 1982). Therefore, behavioural observations during critical periods (adverse weather) likely would have been more representative of duckling behaviour and perhaps provided more

insight into behavioural differences between ducklings with and without transmitters.

. This study indicates that duckling mortality increased with the use of back-mounted transmitters with a subcutaneous anchor compared to unmarked ducklings. Adverse weather likely had an indirect effect on ducklings with transmitters by further increasing energetic costs of thermoregulation through heat loss and plumage disruption. Also, increased energetic costs of ducklings with transmitters result in sublethal effects on growth and weight gain compared to ducklings without transmitters. Despite this, reliable estimates of duckling survival are needed as population models indicate that duckling survival has a substantial effect on population size (Johnson et al. 1992). However, studies that have used transmitters likely over estimate duckling mortality and researchers should be very cautious when interpreting results from these studies. Therefore, more research should be directed to developing a reliable and safe transmitter and attachment method as radio telemetry will likely increase our understanding of mortality factors associated this ducklings

Table A.1. Comparison of mortality in radiomarked and unmarked mallard ducklings in outdoor pens on St Denis National Wildlife Area, Saskatchewan, Canada. Ducklings in the transmitter group received back-mounted transmitters with a subcutaneous anchor during bupivacaine local anesthesia, ducklings in the sham group were given the local anesthetic and control ducklings were handled only. Ducklings in all groups were handled for an equal amount of time and transmitters were placed within 12 hours of hatch.

Year	Treatment	Duckling Fate		
		Died	Lived	Total
1997	Transmitter	5 (29%)[a]	12	17
	Control	1 (5%)	18	19
	Total	6 (17%)	30	36
1998	Transmitter	19 (37%)[b]	32	51
	Sham	4 (7%)	50	54
	Control	2 (4%)	47	49
	Total	25 (16%)	129	154

[a]Fisher's exact test, df = 1, P = 0.049

[b]G-test= 24.026, df = 2, P = 0.001

Table A.2. Mean (± SE) for several growth variables differing significantly between treatment groups ($P \leq 0.05$) using a nested ANOVA for mallard ducklings in 1998. Ducklings in each brood were divided randomly into 3 groups with one third of the brood receiving a transmitter during bupivacaine local anesthesia, one third a sham (including local anesthetic) and one third handled control. Ducklings in all groups were handled for an equal amount of time.

Variable	Significant Effect	Treatment	Mean ± SE
mass slope	treatment	transmitter	0.040 ± 0.002
		sham	0.045 ± 0.001
		control	0.046 ± 0.001
30-day mass	treatment	transmitter	458.5 g ± 8.1
		sham	500.1 g ± 8.0
		control	503.9 g ± 7.0
	sex	female	483.6 g ± 5.4
		male	490.9 g ± 10.3
tarsus length slope	treatment	transmitter	0.009 ± 0.0005
		sham	0.0112 ± 0.0003
		control	0.0116 ± 0.0003
30-day tarsus length	sex	female	42.7 mm ± 0.2
		male	43.8 mm ± 0.2
30-day beak length	sex	female	39.1 mm ± 0.3
		male	40.1 mm ± 0.3
beak width slope	treatment	transmitter	0.0113 ± 0.0004
		sham	0.0120 ± 0.0002
		control	0.0120 ± 0.0001

30-day beak width	treatment	transmitter	18.7 mm ± 0.1
		sham	18.9 mm ± 0.1
		control	19.0 mm ± 0.1
	sex	female	18.7 mm ± 0.1
		male	19.1 mm ± 0.1
head length slope	sex	female	0.0115 ± 0.0001
		male	0.0119 ± 0.0002
30-day head length	treatment	transmitter	85.6 mm ± 0.5
		sham	87.0 mm ± 0.5
		control	87.7 mm ± 0.5
	sex	female	85.6 mm ± 0.3
		male	88.3 mm ± 0.4

Table A.3. Comparison of behaviour (mean ± SE) in radiomarked and unmarked mallard ducklings in outdoor pens on St Denis National Wildlife Area, Saskatchewan, Canada. Behaviour was recorded every minute, and each parameter was calculated as percent time spent performing behaviours as matched groups (marked and unmarked) within each brood. Ducklings in the transmitter group received back-mounted transmitters with a subcutaneous anchor during bupivacaine local anesthesia, ducklings in the sham group were given the local anesthetic and control ducklings were handled only. Ducklings in all groups were handled for an equal amount of time and transmitters were placed within 12 hours of hatch.

		Behaviour (%)						
Year	Treatment	Locomotion	Resting	Foraging	Brooded	Comfort	Out of Sight	Preening Event[a]
1997	Transmitter	33 ± 4	30 ± 3	14 ± 2	0.1 ± 0.1	11 ± 3	12 ± 4	0.6 ± 0.4
	Unmarked	40 ± 3	28 ± 3	17 ± 3	0.2 ± 0.1	5 ± 2	5 ± 2	4.8 ± 0.6
1998	Transmitter	43 ± 4	33 ± 3	16 ± 2	0	9 ± 2	2 ± 1	0.6 ± 0.3
	Unmarked[b]	48 ± 3	27 ± 2	22 ± 2	0.2 ± 0.1	2 ± 0.4	1 ± 0.3	3.5 ±0.5

[a]Preening event was calculated as preening event/duckling ($P < 0.05$)

[b]In 1998, it was impossible to distinguish sham from control ducklings in the unmarked group, therefore behaviour for these groups were pooled.

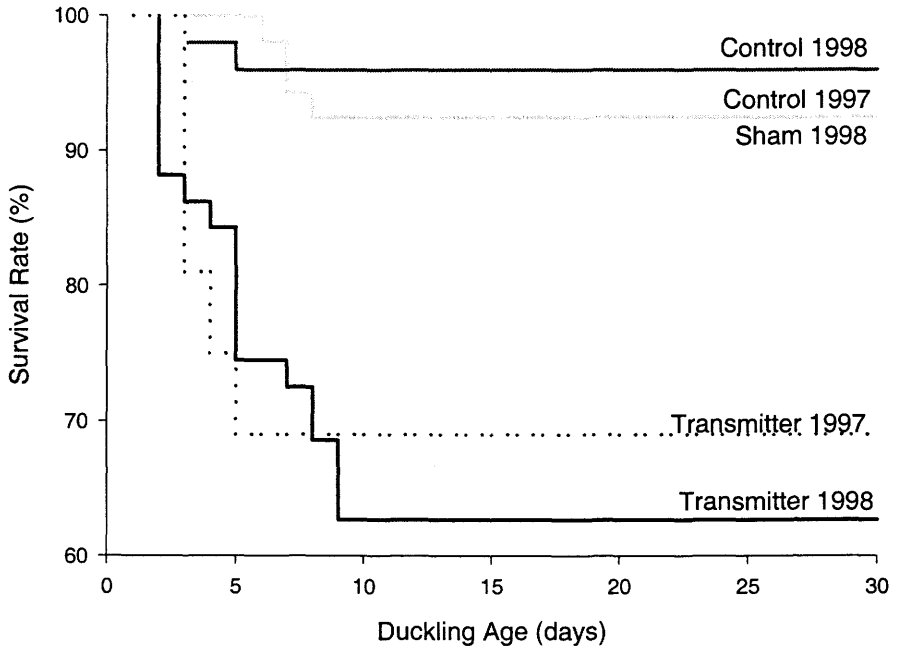

Figure A.1. Survival of radio marked and unmarked mallard ducklings in outdoor pens on St. Denis National Wildlife Area, Saskatchewan, Canada in 1997 and 1998. In 1997, 36 day-old ducklings (5 broods) were divided randomly into 2 matched groups, where half the ducklings in each brood received transmitters and half the ducklings did not. In 1998, 154 day-old ducklings (21 broods) were divided randomly into 3 groups where one third of the brood received a transmitter, one third a sham surgery and one third untreated control. The sham group were treated the same as the transmitter group but no transmitter was placed nor was a skin incision made. All ducklings were handled for an equal amount of time and transmitters were placed within 12 hours of hatch.

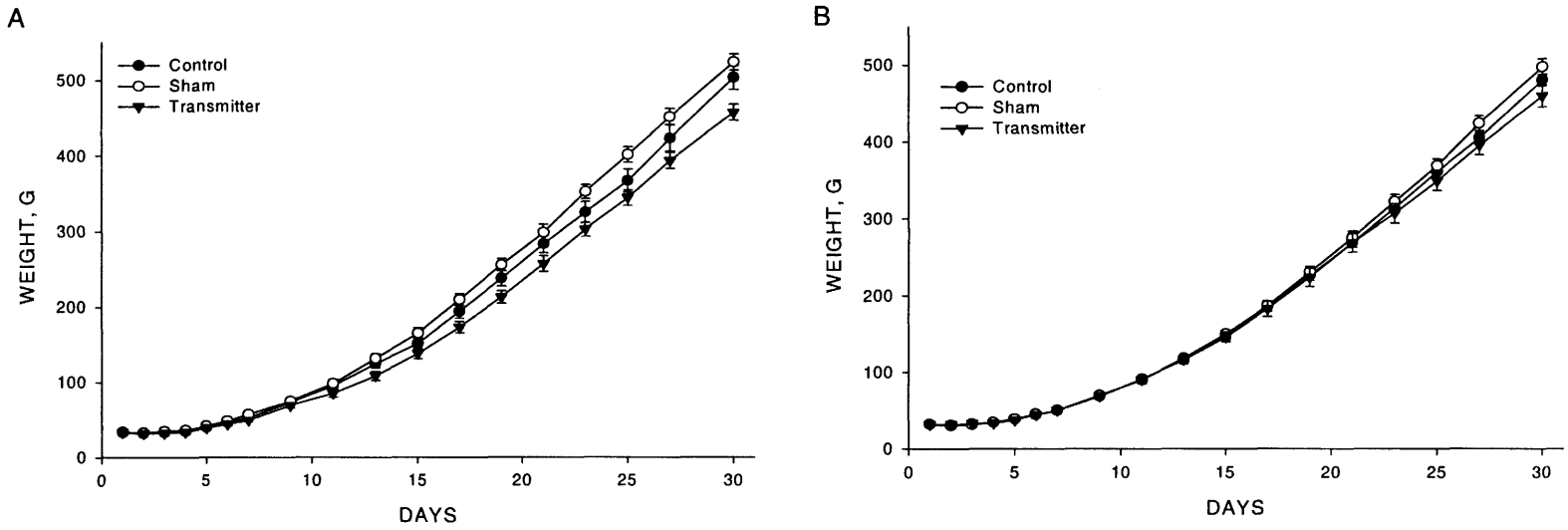

Figure A.2. Mass gain of A) male and B) female mallard ducklings in outdoor pens on St. Denis National Wildlife Area, Saskatchewan, Canada in 1998. Day-old ducklings (154 in 21 broods) were divided randomly into 3 groups where one third of the brood received a transmitter, one third a sham surgery and one third untreated control. The sham group were treated the same as the transmitter group but no transmitter was placed nor was a skin incision made and the control group was handled only. All ducklings were handled for an equal amount of time and transmitters were placed within 12 hours of hatch. Ducklings were weighed every day for the first 7 days followed by every second until day 27 and then on day 30.